CANADIAN JOURNAL OF RESEARCH

VOLUME XV

SECTION D

January to December, 1937

CANADA

Published by the

NATIONAL RESEARCH COUNCIL *of* CANADA

SECTION D

INDEX TO VOLUME XV

Authors

Boyd, E. M.—The interrelation of blood lipids, 1.

Boyd, E. M., Orr, J. H. and Reed, G. B.—Plasma lipids in actively immunized rabbits, 176.

Cameron, T. W. M.—Concepts and mechanisms of resistance in helminthic infections, 77.
Studies on the endoparasitic fauna of Trinidad mammals. V. Further parasites from the Ocelot, 24.
Studies on the heterophyid trematode *Apophallus venustus* (Ransom, 1920) in Canada.
Part II. Life history and bionomics, 38.
Part III. Further hosts, 275.

Griffiths, H. J.—Some observations on the overwintering of certain helminth parasites of sheep in Canada, 156.

Hart, Josephine F. L.—Larval and adult stages of British Columbia Anomura, 179.

LaRocque, A. and Oughton, J.—A preliminary account of the Unionidae of Ontario, 147.

McColl, Ellen L.—See Wardle, R. A.

Miller, M. J.—The experimental infection of pigeons and poultry with *Ascaridia* and *Heterakis*, 105.
The parasites of pigeons in Canada, 91.

Orr, J. H.—See Boyd, E. M.

Oughton, J.—See LaRocque, A.

Parnell, I. W.—Studies on the bionomics and control of the bursate nematodes of horses and sheep. IV. On the lethal effects of some nitrogenous fertilizers on the free-living stages of Sclerostomes, 127.

Peck, O.—The male genitalia in the Hymenoptera (Insecta), especially the family Ichneumonidae.
I. Comparative morphology, 221.
II. Taxonomy of the Ichneumonid genitalia, 253.

Pickett, A. D.—Studies on the genus *Rhagoletis* (Trypetidae) with special reference to *Rhagoletis pomonella* (Walsh), 53.

Putman, W. L.—Biological notes on the Chrysopidae, 29.

Reed, G. B.—See Boyd, E. M.

Richardson, L. R.—*Raphidascaris laurentianus* sp. n. (Ascaroidea) from *Salvelinus fontinalis* (Mitchill) in Quebec, 112.

Stanley, J.—A note on the reduction in the fecundity of *Tribolium confusum* due to eating its own eggs, 111.

Wardle, R. A.—The physiology of the sheep tapeworn, *Moniezia expansa* Blanchard, 117.

Wardle, R. A. and McColl, Ellen L.—The taxonomy of *Diphyllobothrium latum* (Linné, 1758) in western Canada, 163.

SECTION D

INDEX TO VOLUME XV

Subjects

Actinonaias carinata, 152.

Alasmidonta, 152.

Amblema. 152.

Amia calva as host of Apophallus venustus, 275.

Ammonium sulphate, Effect on free-living stages of Sclerostomes, 142.

Anodonta, 152.

Anodontoides, 152.

Anomura
- Comparative morphology of, 193.
- Larvae and adult stages of British Columbia, 179.

Apophallus venustus
- Fish hosts of, 48.
- Snail hosts of, 41.
- Studies on the heterophyid trematode, in Canada
  - II. Life history and bionomics, 38.
  - III. Further hosts, 275.

Ascaridia, Experimental infection of pigeons and poultry with, 105.

Ascaridia columbae
- Artificial infection of fowl with, 108.
- Description of, 95.

Ascaris galli, Artificial infection of pigeons with, 106.

Blood lipids, The interrelation of, 1.

Bowfin (Amia calva), as host of Apophallus venustus, 275.

Calcium cyanamide, Effect on free-living stages of Sclerostomes, 133.

Calcium nitrate, Effect on free-living stages of Sclerostomes, 139.

Calnitro, Effect on free-living stages of Sclerostomes, 138.

Calurea, Effect on free-living stages of Sclerostomes, 132.

Capillaria columbae Rudolphi, Description of, 97.

Caruncul‌ina parva, 152.

Catfish (Ictalurus punctatus) as host of Apophallus venustus, 275.

Cestoda in pigeons, 92.

Chrysopa downesi, 35.

Channel cat (Ictalurus punctatus) as host of Apophallus venustus, 275.

Chrysopidae, Biological notes on the, 29.

Compatibility between helminth and host, 81.

Crustacea, Classification of decapod, 181.

Cryptinae, 254.

Cyclonaias tuberculata, 152.

Diammonium phosphate, Effect on free-living stages of Sclerostomes, 140.

Diphyllobothrium latum in western Canada, The taxonomy of, 163.

Dogfish, Fresh-water (Amia calva), as host of Apophallus venustus, 275.

Dysnomia, 152.

Echinostoma paraulum, Description of, 98.

Elliptio, 152.

Fertilizers, Effect of some nitrogenous, on the free-living stages of sclerostomes, 127.

Fish as hosts of Apophallus venustus (Ransom, 1920) in Canada, 275.

Fusconaia, 152.

Genitalia in the Hymenoptera, The male, 221, 253.

Helminthic infections, Concepts and mechanisms of resistance in, 77.

Helminths
- Factors in successful reproduction of, 78.
- in sheep in Canada, 156.
- of Canadian pigeons, 93.
- Resistance to winter temperatures in Canada, 159.

Heterakis, The experimental infection of pigeons and poultry with Ascaridia and, 105.

Heterakis gallinae, Artificial infection of pigeons with, 107.

Horses, Bionomics and control of bursate nematodes of, 127.

Hymenoptera, The male genitalia in the, 221, 253.

Ichneumonidae
Classification of the, 254.
The male genitalia in the, 221.
Structure and musculature of, 235.

Ichneumoninae, 254.

Ictalurus punctatus as host of Apophallus venustus, 275.

Lampsilis, 152.

Lasmigona, 152.

Leptodea, 152.

Ligumia, 152.

Lipids
Blood, The interrelation of, 1.
Plasma, in actively immunized rabbits, 176.

Longevity of the sheep tapeworm, Moniezia expansa, 119.

Lush (**Amia calva**), as host of Apophallus venustus, 275.

Megarhyssa lunator, Morphology of, 237.

Meleoma emuncta, 36.

Meleoma signoretti, 35.

Micromya, 152. 153.

Moniezia expansa, 156.
The physiology of, 117.

Morphology of the male genitalia in the Hymenoptera, 221.

Nematoda in pigeons, 92.

Nematodes, Studies on the bionomics and control of the bursate, of horses and sheep. IV. On the lethal effect of some nitrogenous fertilizers on the free-living stages of sclerostomes, 127.

Nematodirus filicollis, 156.

Nitro-chalk, Effect on free-living stages of Sclerostomes, 140.

Obliquaria reflexa, 153.

Obovaria, 153.

Ophioninae, 256.

Oriental fruit moth, Influence of Chrysopids on the, 37.

Orthopagurus schmitti, Larval and adult stages of, 179.

Ostertagia circumcincta, 156.

Paguristes turgidus, Larval and adult stages of, 179.

Pagurus beringanus, Larval and adult stages of, 179.

Parasites
Helminth, in sheep in Canada, 156.
of pigeons in Canada, 91.

Pigeons
Experimental infection of, with Ascaridia and Heterakis, 105.
Parasites of, in Canada, 91.

Pimplinae, 255.

Plasma lipids, 2.
in actively immunized rabbits, 176.

Pleurobema, 153.

Potassium nitrate, Effect on free-living stages of sclerostomes, 135.

Poultry, Experimental infection of, with Ascaridia and Heterakis, 105.

Proptera, 153.

Ptychobranchus, 153.

Quadrula, 153.

Rabbits, Plasma lipids in actively immunized, 176.

Raphidascaris laurentianus sp. n. from Salvelinus fontinalis in Quebec, 112.

Red blood cells, Lipids of the, 11.

Rhagoletis, Breeding investigations in, 64.

Rhagoletis (Trypetidae), Studies on the genus, with special reference to R. pomonella (Walsh), 33.

Rhagoletis berberis, 60.

Rhagoletis completa, 61.

Rhagoletis cingulata, 60.

Rhagoletis fausta, 61.

Rhagoletis pomonella, 54.

Rhagoletis ribico

Rhagoletis sauvis, 61.

Rhagoletis tabellaria, 62.

Resistance to helminths, 82.
Mechanism of, 83.
Natural and acquired, 84.

Salvelinus fontinalis, Raphidascarus laurentianus in, 112.

Scolex of Diphyllobothrium latum, 164.

Sclerostomes, Effect of nitrogenous fertilizers on the free-living stages of, 127.

Sheep

Bionomics and control of bursate nematodes of, 127.

Physiology of Moniezia expansa, the tapeworm inhabiting the, 117.

Some observations on the overwintering of certain helminth parasites of, in Canada, 156.

Sodium nitrate, Effect on free-living stages of Sclerostomes, 135.

Speckled trout, Raphidascaris laurentianus in, 112.

Sterna in the Hymenoptera, 227.

Strobila of Diphyllobothrium latum, 164.

Strophitus rugosus, 153.

Tapeworm, Moniezia expansa, The physiology of the sheep, 117.

Taxonomy of the ichneumonid genitalia, 253.

Terga in the Hymenoptera, 224.

Tolerance in helminth parasitism, 88.

Trematoda in pigeons, 91.

Tribolium confusum, A note on the reduction in fecundity of, due to eating its own eggs, 111.

Trichostrongylus colubriformis, 156.

Truncilla, 153.

Tryphoninae, 255.

Unionidae of Ontario, A preliminary account of, 147.

Upogebia pugettensis, Larval and adult stages of, 179.

Urea, Effect on free-living stages of Sclerostomes, 130.

White blood cells, Lipids of the, 18.

Canadian Journal of Research

Issued by The National Research Council of Canada

VOL. 15, SEC. D. JANUARY, 1937 NUMBER 1

THE INTERRELATION OF BLOOD LIPIDS[1]

By Eldon M. Boyd[2]

Abstract

The relation of the concentration of neutral fat, phospholipid, free cholesterol and cholesterol esters of blood plasma, the red blood cells and the white blood cells of man to increasing percentages of total lipid in each of these respectively was determined by oxidative micromethods in a series of more than 900 lipid extracts. A parallel increase in all four lipids was noted with increasing amount of total lipid in plasma until the total lipid exceeded the normal range. The earliest manifestation of a lipemia was a precipitous increase in plasma neutral fat and no increase or a decline in plasma cholesterol esters which later rose again as the lipemia developed. Increasing amounts of total lipid in the red blood cells was found due chiefly to phospholipid; although when the total lipid exceeded the normal range appreciable amounts of neutral fat appeared, the increase in cellular lipids bearing no direct relation to increases in plasma lipids. The same relation was established for the white blood cells as for the red blood cells but the leucocytes contained considerably more lipids than either plasma or the red blood cells.

A correlation has been found between the concentration of the several lipids present in blood in a number of situations. It is beyond the scope of this paper to discuss previous results where only two or three such lipids have been compared. The following is a brief review of previous typical studies in which all or nearly all of the known blood lipids have been considered. In 1921, Bloor (2) described the lipaemias of diabetes mellitus and of persistent hemorrhage as associated with increases in all of the plasma lipids. Similar approximately parallel changes in lipid percentages of plasma or serum were found by Bloor (3) and by Bender and Maynard (1) in relation to changes in diet; by Maynard, Harrison and McCay (26) and by Schaible (31) in relation to lactation; by Man and Peters (25) in relation to diabetic acidosis; by Stoesser and McQuarrie (34) and by Boyd (10) in relation to fever; by Page, Kirk and Van Slyke (30) and by Page and Farr (28) in relation to renal disease; by Boyd (9, 14) and by Boyd and Fellows (19) in relation to pregnancy; and by Boyd (11) in relation to the puerperium. In whole blood a parallelism was noted by Chaikoff, McGavack and Kaplan (23), but Okey and Boyden (27) failed to find one in their studies. Few attempts have been made to correlate changes in the red blood cells; Boyd (10) found a tendency towards parallel changes in fever. The author has examined the lipid content

[1] *Manuscript received October 6, 1936.*

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of the white blood cells in a number of conditions (6–8, 13, 16, 17, 20, 22) but has not hitherto recorded any possible relation of leucocytic lipid values to their total lipid.

The method used in the present work consisted in tabulating according to their total lipid values a large number of complete, differential lipid analyses of plasma, the red blood cells and the white blood cells of human blood. These samples of blood were from persons afflicted with a wide variety of diseases and from subjects who were found to have no condition known to affect the lipid values of blood. The numerous analyses were divided into groups of increasing total lipid values, the mean values of total lipid and component lipids were determined in each group, along with the standard deviations of the means, and an attempt was made to correlate the concentrations of the component lipids with that of the total lipid. In this manner it was possible to ascertain the average part played by each of the component lipids in the development of a lipaemia or of a lipopenia in plasma and to obtain some conception of the relative function of the lipids of the red cells and of the white cells in relation to their total lipid. Wide variations found in the values of certain of the cellular lipids rendered invalid definite conclusions based on comparisons of means. It may be noted that the term, *lipopenia*, was introduced by Boyd (10) to describe the occurrence of a general decrease in blood lipid values.

Oxalated or citrated blood was used and was taken usually from fasting subjects although it was not possible to insist upon fasting conditions in all cases. All of the subjects were on a standard, hospital diet, and under these conditions meals have been shown by Boyd (12) to have little influence upon the concentration of plasma lipids of normal persons although they may influence to some slight extent the lipid content of the red blood cells (21). It has been shown that oxalated, and presumably also citrated, plasma contains roughly 10% less lipid than heparinized or defibrinated plasma (18) while oxalated red blood cells contain proportionately more lipid than do red blood cells from defibrinated blood (15). Extracts were prepared and analyzed by Bloor oxidative micromethods as modified by Boyd (4, 5).

The Lipids of Plasma

The interrelation of blood lipid values will be considered under three main headings, the lipids of plasma, of the red blood cells and of the white blood cells. The interrelation in human blood plasma was determined from 421 complete, differential analyses representing over 4,000 lipid values. The series included 118 differential analyses on normal subjects, the remainder being of abnormal conditions. The lowest plasma total lipid was 100 mg. per cent (milligrams of total lipid per 100 cc. of plasma) and the highest was 1532 mg. per cent. The series was divided into 12 groups, Group 1 including cases with a plasma total lipid up to and including 199 mg. per cent, Group 2 from 200 to 299 mg. per cent, Group 3 from 300 to 399 mg. per cent, etc.

The Interrelation of All Cases

An analysis of these lipid values (approximately 4,000) has been presented statistically in Table I. In this and in succeeding tables and figures, the value of cholesterol ester has been calculated as the sum of ester cholesterol plus

TABLE I

THE LIPID COMPOSITION OF 421 SAMPLES OF HUMAN BLOOD PLASMA GROUPED ACCORDING TO THE VALUE FOR TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS

(The lipid values are expressed in mg. per 100 cc. of blood plasma)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos-pholipid	Free cholesterol	Cholesterol ester
		Minimum	100	13	21	5	13
		Maximum	185	91	90	29	52
100–199	17	Mean	138	42	52	13	31
		Standard deviation	27	20	21	7	13
		Standard deviation in per cent of mean	20%	48%	40%	54%	42%
		Minimum	200	0	28	11	18
		Maximum	298	201	139	39	114
200–299	17	Mean	251	94	74	22	51
		Standard deviation	32	61	36	9	28
		Standard deviation in per cent of mean	13%	65%	48%	41%	55%
		Minimum	303	0	42	6	2
		Maximnm	398	283	191	42	185
300–399	51	Mean	359	102	116	33	108
		Standard deviation	29	47	27	8	35
		Standard deviation in per cent of mean	8%	46%	23%	24%	33%
		Minimnm	404	28	52	28	0
		Maximum	499	270	200	90	245
400–499	82	Mean	458	129	140	43	146
		Standard deviation	28	51	28	10	41
		Standard deviation in per cent of mean	6%	40%	20%	23%	28%
		Minimum	502	0	105	23	97
		Maximum	599	300	235	65	299
500–599	92	Mean	549	145	170	48	186
		Standard deviation	32	49	30	9	39
		Standard deviation in per cent of mean	6%	34%	18%	19%	21%
		Minimnm	602	11	113	27	65
		Maximum	695	437	259	93	419
600–699	60	Mean	648	186	198	55	209
		Standard deviation	28	70	27	13	56
		Standard deviation in per cent of mean	4%	38%	14%	24%	27%
		Minimum	703	90	156	45	66
		Maximum	798	408	285	100	272
700–799	27	Mean	746	254	219	66	207
		Standard deviation	27	71	32	14	53
		Standard deviation in per cent of mean	4%	28%	15%	21%	26%

TABLE I—*Concluded*

THE LIPID COMPOSITION OF 421 SAMPLES OF HUMAN BLOOD PLASMA GROUPED ACCORDING TO THE VALUE FOR TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS

(The lipid values are expressed in mg. per 100 cc. of blood plasma)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos-pholipid	Free cholesterol	Cholesterol ester
800–899	33	Minimum	801	97	151	47	0
		Maximum	896	528	422	136	367
		Mean	847	325	248	76	198
		Standard deviation	30	80	46	21	80
		Standard deviation in per cent of mean	4%	25%	19%	27%	40%
900–999	17	Minimum	901	242	173	60	99
		Maximum	997	570	314	92	389
		Mean	947	341	261	76	269
		Standard deviation	30	82	39	8	63
		Standard deviation in per cent of mean	3%	24%	15%	11%	23%
1,000–1,099	10	Minimum	1012	168	192	40	217
		Maximum	1082	518	352	108	569
		Mean	1053	384	292	81	296
		Standard deviation	24	103	42	18	97
		Standard deviation in per cent of mean	2%	27%	14%	22%	33%
1100–1299	6	Minimum	1103	230	268	95	130
		Maximum	1231	622	336	100	499
		Mean	1142	441	313	98	290
		Standard deviation	42	103	33	2	168
		Standard deviation in per cent of mean	4%	23%	11%	2%	58%
1300 and over	9	Minimum	1329	382	255	90	269
		Maximum	1532	773	510	123	481
		Mean	1401	540	393	107	361
		Standard deviation	65	116	75	10	68
		Standard deviation in per cent of mean	5%	21%	19%	9%	18%

cholesterol ester fatty acid as appeared in previous presentations of results (*e.g.*, 5). The method of determining and calculating the several lipids listed in these figures and tables has been previously described (5). In offering the results in this manner, the author has had in mind the simplification of description of data on blood lipids. As blood lipids are understood at present, there are four main groups; namely, the neutral fats, phospholipids, cholesterol esters and free cholesterol. Such values as ester cholesterol, total cholesterol, total fatty acids, etc., are convenient laboratory expressions since they represent values that may be determined by methods of analysis. When only part of a complete lipid analysis is performed, these laboratory values must needs be reported. When different methods of analysis are compared, it is also preferable to compare experimentally determined rather than calculated values. But for purposes of description such as is aimed at herein, these laboratory expressions may well be omitted. Such additional data may be calculated if desired, from the information given in the several tables, by applying the factors previously discussed (5).

The statistical analysis of results presented in Table I includes the listing of the minimal value for each lipid in each of the groups, the corresponding maximal value, mean value, standard deviation of the mean and, for purposes of comparing relative variations, the percentage standard deviation or the standard deviation expressed as a percentage of the mean. The standard deviation was calculated from the formula:

$$\varphi = \sqrt{\Sigma(x)^2/n}$$

where φ represents the standard deviation, Σ a summation symbol, x the difference between each individual value and the mean value and n the total number of values considered in the mean. To obtain the value of the standard deviation, each individual value was subtracted from the mean, these differences were squared, added together, divided by the total number of values and the square root of the resulting figure gave the standard deviation. From the mean and the standard deviation the expected range of values may be calculated. Thus two-thirds of values may be expected to lie within the range of the mean plus and the mean minus the standard deviation while 95% of all expected cases lie within the range of the mean plus and minus twice the standard deviation.

There was comparatively little variation in the values for *total lipid* in each of the groups given in Table I. The greatest relative variation occurred in the first group of 100–199 mg. per cent total lipid. In this group the mean was 138 mg. per cent and the standard deviation 27, which was 20% of the mean. The percentage standard deviation declined to 13% in the second group, to 8% in the third group, to 6% in the fourth group, and from then on it remained at low values between 2 and 5%. The percentage standard deviation for total lipid was lower in all except the first two groups than the percentage standard deviation of practically all the other lipids. The reason for this was that total lipid values were selected within a certain range and hence were not subject to the same chance variation as the other lipids. The standard deviations for the means were approximately the same in most of the total lipid groups, and since the means for total lipid became progressively greater, the percentage standard deviation obviously had to become progressively less.

Values for *neutral fat* were found to be amongst the most variable of all plasma lipids. The standard deviation may be seen from Table I to extend from 21 to 65% of the respective means. The neutral fat of human plasma was found to range from none at all to as high as 773 mg. per cent. In one group the range of values for neutral fat was from 0 to 201 mg. per cent although the total lipid figures of this group covered a range of only 98 mg. per cent. Neutral fat may enter plasma either from the tissues which store fat (neutral fat is the chief type of storage fat) or from the intestine during fat absorption. Part of it at least is carried to the liver where it is probably converted into phospholipid and cholesterol esters. During extensive parenchymatous damage to the liver, the author has noted a marked decrease in the phospholipid and cholesterol ester content of plasma with neutral fat

values as great as, or greater than, normal, suggesting that in this condition the liver failed to convert the neutral fat coming to it into phospholipid and cholesterol esters.

The concentration of plasma *phospholipid* extended from 21 to 510 mg. per cent in the series of cases studied. There was less variation in the phospholipid content in the various groups than occurred in neutral fat. The standard deviations extended from 11 to 48% of the respective means, the greatest variation being again encountered in those groups with lowest mean values. One function of plasma phospholipid has been definitely shown by Sinclair (32) to be the transport of fatty acids. Boyd and Wilson (22) showed that phospholipids transport fat from the placenta to the human foetus *in utero*.

The *free cholesterol* content of plasma covered a range of from 5 to 136 mg. per cent. The standard deviation varied between 2 and 54% of the means, being greatest in the groups with lowest mean values. This fraction is usually designated "free" cholesterol to distinguish it from "ester" or "combined" cholesterol and from the sum of these, the "total" cholesterol. It would appear preferable to designate free cholesterol merely "cholesterol" and the other fraction "ester cholesterol" since the nomenclature in common usage has tended to imply that the only function of plasma cholesterol is its role in fat metabolism, and to render obscure the fact that cholesterol may, and probably does, have other important functions such as, for example, the possible relation of cholesterol to the genesis of sex hormones. At present, when merely blood cholesterol is stated, the inference is that blood total cholesterol is meant. Cholesterol is related to lipid metabolism in that it forms esters with fatty acids and it would appear, as previously stated, that this synthesis is effected at least in the liver. Sperry (33) has shown that there is in blood plasma a cholesterol esterase which splits cholesterol esters.

Values for plasma *cholesterol ester* were comparable in their variability to those of neutral fat. The concentration of this lipid extended from 0 to 569 mg. per cent and the standard deviation ranged from 18 to 58% of the respective means. Part at least of the variation in cholesterol ester values is due to the fact that this lipid is calculated from two or more experimentally determined figures, and hence its value would tend to contain a summation of experimental errors. Cholesterol ester is sometimes written in the singular, sometimes in the plural. While there is apparently only one fatty acid group attached to one molecule of cholesterol in the ester, there is little doubt but that in plasma there are many different compounds of cholesterol with various fatty acids.

The Relation of Component Lipids to Total Lipid of Plasma

In Fig. 1 the mean values of the individual lipids listed in Table I have been plotted against the mean values of the total lipid. The relation of one lipid to another in the lipopenic, normal or lipaemic phases may thus be clearly seen. This figure demonstrates that the three chief lipids of plasma are cholesterol esters, neutral fats and phospholipids and that the fourth

so-called lipid, namely, free cholesterol, is present in much smaller concentrations throughout. In order to give some conception of the relation of these curves to the normal range of values, a "normal range" has been indicated in Fig. 1. This "normal range" is the expected range of 95% of values for total lipid of normal human adults as taken from Table III. The values of the several curves passing through the normal range in Fig. 1 are not necessarily normal values for these component lipids, since the curves are drawn from mean values of both normal and abnormal values. The relation of normal values for component lipids to their total lipid will be considered later.

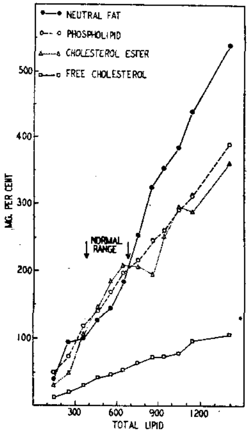

FIG. 1. *The relation of mean values of lipids in blood plasma of healthy and diseased persons to mean values of plasma total lipid expressed in mg. per 100 cc.*

Fig. 1 illustrates that the concentration of phospholipid and cholesterol ester follow an almost parallel course from the lowest lipopenic values to about the upper limit of the normal range of total lipid. Beyond this point, phospholipid continued to increase at much the same rate, bearing almost a direct linear relation to total lipid throughout. Cholesterol ester passed through a temporary declining or stationary phase when it reached the upper limit of the normal range, this phase persisting from average total lipid values of from about 600 to 900 mg. per cent. It may be inferred that the onset of a lipaemia is accompanied by a temporary decline in plasma cholesterol ester values. This peculiar behavior of cholesterol esters is emphasized because it has been recorded in the results of Gardner and Gainsborough (24) and, in somewhat modified form, in the results of Boyd and Fellows (19) in their studies of blood lipids in pregnancy of women and of guinea pigs. Such a phase in cholesterol ester values has not been stressed in previous considerations of the different types of lipaemia wherein it has apparently been missed by insufficiently detailed study. What its significance may be is at present obscure. It indicates that there must be some important difference in the metabolism of this lipid in contrast to the others and refutes the conception (26) that all lipid values of plasma increase at the same rate and at the same time. Following the phase of decline, the cholesterol ester content of plasma increased roughly parallel to that of phospholipid, with the exception of a further decline at total lipid values of about 1000 to 1100 mg. per cent. The second decline may be an artifact or it may indicate a tendency for fluctuation in plasma cholesterol ester values such as are found in hog cholera and in the

lipopenia of fever in man (10), fluctuations which again have not been stressed in most other considerations of plasma cholesterol ester. It is apparent that much detailed study of the changes in the concentration of this lipid in particular and of all lipids in general is necessary before explanations as to their functions should even be attempted.

Values for free cholesterol exhibited, like those of phospholipid, a direct linear relation to total lipid. The similarity in behavior of these two lipids is further evidence in support of the hypothesis that they are closely allied in vital economy, the one being considered the antagonist of the other.

The Interrelation of Plasma Lipids in Normal Cases

In Table II have been listed values for plasma lipids in 118 samples of blood from normal human adults grouped according to the concentration of total lipid. The lowest figure for total lipid encountered in this series was

TABLE II

THE LIPID COMPOSITION OF 118 SAMPLES OF BLOOD PLASMA FROM NORMAL HUMAN ADULTS GROUPED ACCORDING TO THE VALUE FOR TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL LIPID ANALYSIS

(The lipid values are expressed in mg. per 100 cc. of plasma)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
To 449	13	Minimum	381	28	98	32	102
		Maximum	447	118	186	56	188
		Mean	427	79	150	41	157
		Standard deviation	20	26	23	10	21
		Standard deviation in per cent of mean	5%	33%	15%	24%	13%
450–499	26	Minimum	451	50	97	32	109
		Maximum	499	188	200	62	245
		Mean	482	115	156	44	177
		Standard deviation	13	35	38	11	51
		Standard deviation in per cent of mean	3%	30%	24%	25%	29%
500–549	36	Minimum	502	55	111	23	104
		Maximum	546	223	212	64	219
		Mean	522	139	175	44	172
		Standard deviation	14	42	27	8	28
		Standard deviation in per cent of mean	3%	30%	15%	18%	16%
550–599	16	Minimum	551	85	105	35	132
		Maximum	593	213	210	63	284
		Mean	573	164	164	48	197
		Standard deviation	17	38	31	8	38
		Standard deviation in per cent of mean	3%	23%	19%	17%	19%
600–649	18	Minimum	602	125	113	27	65
		Maximum	645	437	228	88	254
		Mean	623	185	181	51	206
		Standard deviation	15	74	25	18	47
		Standard deviation in per cent of mean	2%	40%	14%	35%	23%
Over 649	9	Minimum	650	33	124	38	172
		Maximum	752	317	236	74	419
		Mean	683	202	187	53	241
		Standard deviation	34	81	33	13	70
		Standard deviation in per cent of mean	5%	40%	18%	25%	29%

381 mg. per cent and the highest 752 mg. per cent. The variation of total lipid values, as seen from the percentage standard deviation, was lower than that shown in Table I, which was to be expected since the results given in Table II were from a more homogeneous group of subjects. Plasma neutral fat extended from 28 to 437 mg. per cent and its standard deviation from 23 to 40% of the respective means, this lipid being the most variable in value of all lipids in the plasma of normal human adults. Phospholipid was the least variable of the component plasma lipids, the standard deviation ranging from 14 to 24% of the means. The free cholesterol values were almost as constant as those of phospholipid, the standard deviation extending from 17 to 35% of the respective means. Cholesterol esters extended from 102 to 415 mg. per cent and the standard deviations varied between 13 and 29% of the means.

For purposes of comparison with the preceding curves for both normal and abnormal lipid values in Fig. 1, a similar plotting of mean values against total lipid of normal subjects only has been offered in Fig. 2. It may be seen that the component lipid values of normal human plasma bore a relation to total lipid similar to that of all lipid values within the normal range of total lipid indicated in Fig. 1. The most notable differences were, first, a tendency for neutral fat to increase at a rate greater than that of the other lipids over the whole normal range of total lipid; and second, the concentration of cholesterol esters, although exhibiting fluctuations, did not enter the phase of decline as early as that seen in the plotting of both normal and abnormal cases in Fig. 1. These comparisons would suggest that with further study it may be possible to predict the imminent possibility of a lipaemia developing by a lipid analysis of plasma which may show a normal total lipid, a relatively high neutral fat and a normal or even low cholesterol ester, suggesting that a lipaemia is impending. The close parallelism between phospholipid and free cholesterol is even more evident in Fig. 2 than in Fig. 1. The data presented in Fig. 2 resemble a similar presentation of normal values by Page, Kirk, Lewis, Thompson and Van Slyke (29) in their Fig. 9. Their values for neutral fat are higher than those shown in Fig. 2 and Table II because their method of analysis gave higher values than the oxidative method.

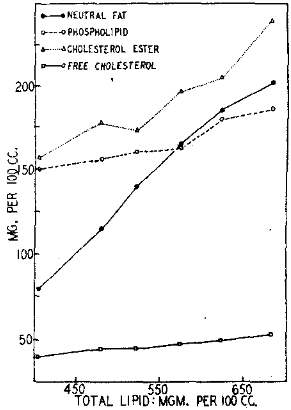

FIG. 2. *The relation of mean values of lipids in blood plasma of normal healthy human adults to mean values of total lipid expressed in mg. per 100 cc.*

The Normal Range of Plasma Lipids

The analyses of the plasma of healthy human adults have been considered as a whole in Table III in order to establish the normal range of lipids by oxidative micromethods based upon a large number of observations. These subjects were of both sexes, and a tabulation of results according to sex

TABLE III

THE LIPID COMPOSITION OF BLOOD PLASMA FROM 118 DIFFERENTIAL ANALYSES ON NORMAL HUMAN ADULTS

(The results are expressed in mg. per 100 cc. of plasma)

Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
Minimum values*	381	28	97	23	65
Maximum values*	752	437	236	88	419
Mean values	530	142	165	46	177
Standard deviation	74	60	28	8	41
Standard deviation in per cent of mean, %	14	42	17	17	23
Expected range of 2/3 of normal subjects	*456–604*	*82–202*	*137–193*	*38–54*	*136–218*
Expected range of 95% of normal subjects	382–678	22–262	109–221	30–62	95–259

**The sum of the individual lipids does not equal the total lipid because the individual lipid values are taken from different analyses.*

indicated that there were no significant differences between the lipid values of plasma of men and of women, providing that blood was not taken during menstruation, the climacteric, or pregnancy in women. The age of the subjects listed extended from 15 to 78 and within this range increasing age did not affect the concentration of plasma lipids. Below the age of 15 and especially in infancy and the newborn there was found a lipopenia but such results have not been included in the tabulation of normal values for healthy human adults. The author has previously reported normal plasma lipid values in occasional small groups of cases (5, 9, 12). These are included with many additional values in Table III. This larger group of normal cases revealed means similar to those previously found, except that the values were somewhat lower, especially for phospholipid.

The minimal and maximal values found for plasma *total lipid* were 381 and 752 mg. per cent. The extremes, which may be calculated to include 95% of all expected values, were 382 and 678 mg. per cent. The mean total lipid was 530 mg. per cent and the standard deviation of the mean was 74. In other words human plasma contains roughly from 0.4 to 0.7% of fatty substances or about the same as the amount of inorganic salts present and much more than the water soluble organic compounds such as the nitrogenous derivatives and glucose. In relation to amount present, the lipids of plasma are exceeded only by the albumins and globulins and are about equal in concentration to that of fibrinogen. The relation of this relatively large con-

centration of lipids to colloidal osmotic pressure of plasma and to the genesis of oedema has not received the study it should warrant, owing to its being in colloidal solution.

The total lipid of plasma was composed, on the average, of 33% of cholesterol esters, 31% of phospholipids, 27% of neutral fats and 9% of free cholesterol. The lipid most variable in amount may be seen from Table III to be neutral fat which yielded an expected range for 95% of expected values between 22 and 262 mg. per cent, a mean of 142 mg. per cent and a standard deviation of 60. The variability of cholesterol esters was smaller than that of neutral fat but greater than that of phospholipid and free cholesterol. The minimal value found for cholesterol esters was 65 and the maximal 419 mg. per cent, the mean 177 mg. per cent, the standard deviation 41 and the expected range of 95% of cases was calculated to be from 95 to 259 mg. per cent. Phospholipid and free cholesterol possessed the same relative variation, the standard deviations being 17% of each of the respective means.

The expected range of two-thirds and of 95% of normal subjects have both been included in Table III. The range of 95% of cases should be used when interpreting the significance of a single analysis, the range of two-thirds of cases when comparing a group of values with the normal. It is rare to find the expected range of two thirds of cases of any condition exhibiting a lipaemia or a lipopenia to be much beyond the expected range of two thirds of normal subjects. Any group of values not thus overlapping in its expected two thirds range the corresponding range of normal subjects may be considered to have demonstrated a significant difference from normal. Actually the correct statistical conclusion to be drawn in such a case is that the expected range of two thirds of all cases of the values in the condition under study may be considered to lie beyond the expected range of two thirds of normal values.

Lipids of the Red Blood Cells

The Interrelation of All Cases

A statistical analysis of the data obtained form 307 complete, differential lipid analyses of human red blood cells of normal and of pathological cases grouped according to the concentration of total lipid of each complete analysis has been presented in Table IV. These results have been condensed from

TABLE IV

THE LIPID COMPOSITION OF 307 SAMPLES OF HUMAN RED BLOOD CELLS GROUPED ACCORDING TO THE VALUE FOR TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS

(The lipid values are expressed in mg. per 100 cc. of red blood cells)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
		Minimum	300	0	156	69	0
		Maximum	399	104	292	128	43
300–399	7	Mean	354	40	209	94	11
		Standard deviation	51	39	46	22	15
		Standard deviation in per cent of mean	14%	98%	22%	23%	136%

TABLE IV—*Concluded*

THE LIPID COMPOSITION OF 307 SAMPLES OF HUMAN RED BLOOD CELLS GROUPED ACCORDING TO THE VALUE FOR TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS—*Concluded*

(The lipid values are expressed in mg. per 100 cc. of red blood cells)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos-pholipid	Free cholesterol	Cholestero ester
400–449	13	Minimum	404	0	191	58	0
		Maximum	446	169	344	118	74
		Mean	431	26	296	93	16
		Standard deviation	13	46	40	19	27
		Standard deviation in per cent of mean	3%	177%	14%	20%	169%
450–499	65	Minimum	450	0	198	62	0
		Maximum	499	110	391	179	99
		Mean	477	38	301	106	32
		Standard deviation	16	35	38	18	24
		Standard deviation in per cent of mean	3%	92%	13%	17%	75%
500–549	63	Minimum	501	0	109	73	0
		Maximum	546	262	417	150	147
		Mean	527	62	313	111	41
		Standard deviation	22	48	51	19	34
		Standard deviation in per cent of mean	4%	77%	16%	17%	83%
550–599	49	Minimum	550	0	231	68	0
		Maximum	595	233	453	220	182
		Mean	573	52	353	128	40
		Standard deviation	14	54	50	28	40
		Standard deviation in per cent of mean	2%	104%	14%	22%	100%
600–649	25	Minimum	600	0	284	79	0
		Maximum	646	191	478	173	230
		Mean	627	64	384	126	53
		Standard deviation	14	59	34	22	27
		Standard deviation in per cent of mean	2%	92%	9%	17%	51%
650–699	23	Minimum	651	0	237	85	0
		Maximum	692	279	540	189	172
		Mean	670	63	430	136	41
		Standard deviation	14	78	68	23	53
		Standard deviation in per cent of mean	2%	124%	16%	17%	129%
700–799	21	Minimum	705	0	230	55	0
		Maximum	792	304	574	192	137
		Mean	743	153	422	138	30
		Standard deviation	28	101	84	33	48
		Standard deviation in per cent of mean	4%	66%	20%	24%	160%
800–999	21	Minimum	801	0	318	87	0
		Maximum	999	497	698	206	221
		Mean	882	216	467	141	58
		Standard deviation	39	142	89	27	61
		Standard deviation in per cent of mean	4%	66%	19%	19%	105%
Over 1000	18	Minimum	1000	0	272	50	0
		Maximum	1894	1380	864	217	391
		Mean	1266	557	502	117	90
		Standard deviation	298	426	170	43	116
		Standard deviation in per cent of mean	23%	77%	34%	37%	129%

more than 3,000 lipid values. The data were divided into 10 groups of increasing total lipid values as indicated in the first column of Table IV. Although presented in a manner similar to those of plasma, it should not thus be inferred that the lipid values of the red blood cells bore a direct relation to the lipid content of plasma. It should not be inferred, in other words, that as the lipid content of plasma increased, so necessarily did the lipid content of the red blood cells. As a matter of fact such a correlation was seldom found.

The *total lipid* content of the red blood cells varied between 300 and 1894 mg. per cent. As in plasma, the percentage standard deviation was greatest in the groups with lowest total lipid values, except in the last group which included a wide range of very high values. The standard deviation varied from 2% to 23% of the respective means for total lipid. The total lipid content of the red blood cells is approximately the same in amount as that of plasma, covering likewise a range of values similar to that of plasma. There is, however, a marked difference in the composition of the total lipid of the erythrocytes from that of plasma. The lipids being in colloidal solution or bound in the structural make-up of the cell, are not readily diffusible across the red cell membrane and changes frequently occur in the percentage of plasma lipids without being reflected by corresponding changes in those of the red blood cells. The correct degree of such changes is not evident when whole blood is selected for lipid analysis, as is unfortunately often done. There are comparatively few situations in which an analysis of whole blood for lipids can be justified as preferable to an analysis of plasma or of serum. In any thorough study both plasma and the red blood cells should be examined.

Phospholipid may be seen from Table IV to be the lipid of greatest bulk in the red blood cells, and it is believed that this phospholipid is composed chiefly of cephalins and sphingomyelins. The concentration of phospholipid in the several groups was relatively constant, the standard deviation varying from 9 to 34% of the means. It would seem that most of the phospholipid of the red blood cells is structural rather than metabolic in function. Boyd and Tweddell (21) found no diurnal variation in the phospholipid content of the red cells, and Sinclair (32) was unable to detect elaidic acid in the phospholipid fatty acids of the red blood cells of cats after feeding this special type of fat.

Free cholesterol is, next to phospholipid, the lipid of greatest bulk in the red blood cells. The values of free cholesterol were somewhat more variable than those of phospholipid but less variable than those of neutral fat and cholesterol esters. The standard deviation ranged from 17 to 37% of the means. Free cholesterol, like phospholipid, is believed to be largely structural rather than metabolic in function in the red blood cells.

Values for *neutral fat* (Table IV) may be seen to have been found extremely variable. In every group, no matter what the value of total lipid, at least one analysis was recorded in which the neutral fat content was zero. The maximum values in each of the groups ranged from 100 to 300 mg. per cent,

except in the last two groups in which several cases with very high neutral fat values were encountered. The standard deviation extended from 66 to 177% of the means. It is obvious, therefore, that one should interpret with caution any relation between mean values for total lipid and mean values for neutral fat of the red blood cells. There is some evidence that the neutral fat of the red blood cells takes part in the lipid metabolism of the body (21).

The variations in the *cholesterol ester* content of the red blood cells were analogous to those of neutral fat (Table IV). In every group some analyses were found in which the cholesterol ester content was zero, although other members of the group had cholesterol ester values as high as from 43 to 391 mg. per cent. The standard deviation varied from 51 to 169% of the respective means. Thus one should again be cautious with cholesterol ester as with neutral fat in deciding upon any relation between mean values of this lipid and mean values of total lipid. Boyd and Tweddell (21) record evidence suggesting a diurnal variation in the cholesterol ester content of the red blood cells which would suggest that this lipid may also be concerned in sterol and lipid metabolism.

The Relation of Component Lipids to Total Lipid of the Red Blood Cells

In Fig. 3 the mean values for the component lipids in the several groups listed in Table IV have been plotted against the mean values of the total lipid. As in Fig. 1, the expected range of 95% of normal total lipid values has been indicated. The values of the component lipids as they pass through the normal range of total lipid shown in Fig. 3 are not necessarily normal values for these lipids. The normal values are shown in relation to total lipid in Fig. 4.

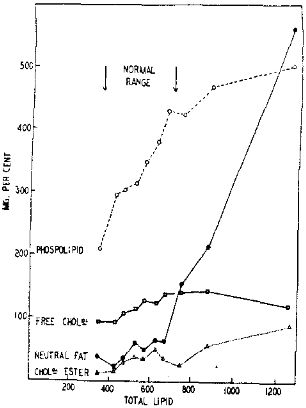

FIG. 3. *The relation of mean values of lipids in the red blood cells of healthy and diseased persons to mean values of total lipid expressed in mg. per 100 cc.*

Fig. 3 clearly demonstrates that the two chief lipids of the red blood cells under normal conditions, namely, phospholipid and free cholesterol, bear a similar relation to total lipid. The rate of increase for phospholipid was greater than that of free cholesterol as the total lipid increased. Values for both lipids bore an approximately direct linear relation to total lipid values until the total lipid exceeded about 900 mg. per cent, after which the increase was less for phospholipid and the free cholesterol content actually showed a decrease.

The marked increase in neutral fat of the red blood cells beyond the upper limit of the normal range of total lipid is due, as is seen from the results in Table IV, to the occurrence of many exceptionally high values in the analyses within the high groups. At the same time other samples of erythrocytes were found to contain small amounts of neutral fat or none at all in these same groups in which very high values occurred. The obvious conclusion from Fig. 3 that the neutral fat content of the red cells is markedly increased when the total lipid content rises very high, cannot therefore be made without reservation. Rather should it be said that with very high total lipid values in the red blood cells one may meet with exceptionally high neutral fat values but that such need not necessarily be found.

A similar situation prevailed with cholesterol ester values. Within the limits of the normal range of total lipid the mean values of cholesterol ester were low but tended to increase with increase in total lipid. There was an interesting analogy between the curve for cholesterol ester in Fig. 3 and the corresponding curve of Fig. 1, in that at and just above the upper limit of the normal range of total lipid, the curve for cholesterol ester of the red cells declined temporarily as it did in plasma. In view of the wide variation in values averaged in the mean and as given in Table IV, it is not possible to attach more than coincidental significance to these similarities. The increase in the mean values of cholesterol esters above the normal range cannot be taken as unqualified evidence that cholesterol ester always increases with increase in total lipid because of the likewise marked variation that was found at this time. It may be stated that high values for cholesterol ester are often found, but not necessarily so, when the lipid content of the red blood cells rises above the normal range.

The Interrelation of Lipids of the Red Blood Cells in Normal Cases

Sixty-six samples of red blood cells from normal human adults were examined. The results obtained have been divided into six groups according to the total lipid values, and are presented in Table V. Variations in the

TABLE V

THE LIPID COMPOSITION OF 66 SAMPLES OF RED BLOOD CELLS FROM NORMAL HUMAN ADULTS GROUPED ACCORDING TO THE VALUE OF TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS

(The lipid values are expressed in mg. per 100 cc. of red blood cells)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
400–449	4	Minimum	425	0	239	90	12
		Maximum	442	71	302	103	66
		Mean	435	21	280	95	49
		Standard deviation	9	29	23	5	33
		Standard deviation in per cent of mean	2%	138%	8%	5%	67%
450–499	13	Minimum	454	0	243	79	0
		Maximum	493	110	293	116	99
		Mean	477	68	264	97	48
		Standard deviation	14	33	32	14	26
		Standard deviation in per cent of mean	3%	49%	12%	14%	54%

TABLE V—*Concluded*

THE LIPID COMPOSITION OF 66 SAMPLES OF RED BLOOD CELLS FROM NORMAL HUMAN ADULTS GROUPED ACCORDING TO THE VALUE OF TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS—*Concluded*

(The lipid values are expressed in mg. per 100 cc. of red blood cells)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos-pholipid	Free cholesterol	Cholesterol ester
500–549	29	Minimum	501	0	220	73	0
		Maximum	549	144	334	138	112
		Mean	528	64	297	105	62
		Standard deviation	17	40	43	17	30
		Standard deviation in per cent of mean	3%	63%	14%	16%	48%
550–599	7	Minimum	552	11	231	68	0
		Maximum	597	167	437	134	110
		Mean	567	65	357	114	31
		Standard deviation	15	49	64	20	38
		Standard deviation in per cent of mean	3%	75%	18%	18%	123%
600–699	7	Minimum	634	54	237	129	0
		Maximum	692	279	418	189	55
		Mean	665	151	330	151	33
		Standard deviation	18	63	53	19	24
		Standard deviation in per cent of mean	3%	42%	16%	13%	73%
700–800	6	Minimum	709	13	274	144	0
		Maximum	789	302	452	192	135
		Mean	736	166	395	163	12
		Standard deviation	32	90	61	18	54
		Standard deviation in per cent of mean	4%	54%	15%	11%	450%

total lipid values were low in all groups. The standard deviation varied from 2 to 4% of the respective means for total lipid. The three lower groups contained zero values for neutral fat and in all groups the minimal value of this lipid was low. The standard deviation in the neutral fat values ranged from 42 to 138% of the respective means, a variation again sufficiently great to render dubious deductions regarding the relation of neutral fat to total lipid. The variations in the phospholipid values were much lower, the standard deviation extending from 8 to 18% of the means. The standard deviation represented 5 to 18% of the means for free cholesterol and hence mean values for both free cholesterol and for phospholipid may be taken as indicative of the trend of the values generally. An extremely wide range of values was recorded for cholesterol esters, the standard deviation being as phenomenally great as 450% of the mean in one group, showing that the means could hardly be regarded as indices for even a majority of values in a group.

With these variations in mind, Fig. 4 may be considered. In this figure the average values for the component lipids in each group have been compared with the means of the total lipid. Since the means for phospholipid and free

cholesterol may be taken as accurate indices of the changes taking place in the majority of samples, it is apparent that phospholipid and free cholesterol parallel each other in changes in concentration which bear roughly a direct linear relation to changes in total lipid. One cannot add from Fig. 4 any further deduction regarding the relation of neutral fat and cholesterol ester to total lipid other than has been expressed above, because of the marked variation in the values averaged in the means. On comparing Fig. 3 with Fig. 4 it is evident that the values of the component red cell lipids of both normal and of abnormal cases follow the same general course when passing through the range of normal total lipid values.

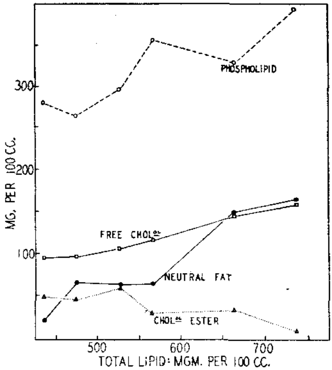

FIG. 4. *The relation of mean values of lipids in the red blood cells of normal healthy human adults to mean values of total lipid expressed in mg. per 100 cc.*

The Normal Range of Lipids in the Red Blood Cells

To establish the normal range of values for lipids in the red blood cells of healthy normal human adults, as estimated by oxidative micromethods, the results in Table VI are offered. The minimal total lipid value encountered in the 66 cases studied was 425 mg. per cent and the highest 789 mg. per cent

TABLE VI

THE LIPID COMPOSITION OF THE RED BLOOD CELLS IN 66 COMPLETE DIFFERENTIAL ANALYSES ON NORMAL HUMAN ADULTS

(The results are expressed in mg. per 100 cc. of red blood cells)

Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
Minimum values*	425	0	220	68	0
Maximum values*	789	302	452	192	135
Mean values	523	84	311	115	13
Standard deviation	86	63	57	27	15
Standard deviation in per cent of mean, %	16	75	18	23	112
Expected range of 2/3 of normal subjects	437–609	21–147	254–368	88–142	0–28
Expected range of 95% of normal subjects	351–695	0–210	197–425	61–169	0–43

**The sum of the individual lipids does not equal the total lipid because the individual lipid values are from different analyses.*

with a mean of 523 mg. per cent and a standard deviation of 86, which was 16% of the mean. The range of 95% of total lipid values for the red blood cells was practically identical to that found in plasma, *i.e.*, roughly from 0.4 to 0.7%.

Total lipid of the normal human red blood cells was composed, on the average, of 59.5% of phospholipid, 22% of free cholesterol, 16% of neutral fat and 2.5% of cholesterol esters. The lipid composition of the red blood cells is thus considerably different from that of plasma. The red cells contain more phospholipid and free cholesterol and less neutral fat and cholesterol esters than does plasma. The average value for red cell neutral fat was 84 mg. per cent, the standard deviation being 63 which was 75% of the mean, and the expected range of 95% of cases was from 0 to 210 mg. per cent. The expected range of 95% of cases for phospholipid was 197 to 425 mg. per cent, for free cholesterol 61 to 169 mg. per cent and for cholesterol ester 0 to 43 mg. per cent.

The Lipids of the White Blood Cells

The Interrelation of All Cases

In Table VII have been assembled the results of 174 complete, differential lipid analyses of human white blood cells, representing about 2,000 lipid values, grouped according to the concentration of total lipid. The lipid content of the white blood cells has been previously reported in a number of conditions (4, 6-8, 13, 16, 17, 20, 22) but has not previously been considered in relation to total lipid content. The results given in Table VII are expressed in mg. per 100 gm. of leucocytes, moist weight, and not in terms of mg. per 100 cc. of blood so that the lipid content of the leucocytes does not necessarily vary with the total leucocyte count.

There was little variation in the concentration of leucocytic total lipid, the standard deviations extending from 5 to 13% of the respective means of the several groups. Of the component lipids, phospholipid was most constant in value, the standard deviation being 19 to 43% of the means. The next least variation was in free cholesterol values, the percentage standard deviation varying from 21 to 38%. Neutral fat was quite variable with standard deviations extending from 63 to 125% of the means while cholesterol esters were most variable, the standard deviation ranging from 98 to 240% of the respective means.

Neutral fat and cholesterol ester have been shown to be the most variable of all lipids in each of plasma, the red blood cells and the white blood cells. A good deal of this variation is probably due to the fact that neither of these lipids are estimated directly but rather from two or more experimental figures. Cholesterol ester is calculated as the sum of ester cholesterol plus cholesterol ester fatty acid, which is calculated as 67% of ester cholesterol and this, in turn, is derived by subtracting the value of free cholesterol from that of total cholesterol, both of which are directly estimated. Neutral fats are calculated as 100/95 times the neutral fat fatty acids which are found by subtracting the values of the phospholipid and cholesterol ester fatty acids from that of

TABLE VII

THE LIPID COMPOSITION OF 174 SAMPLES OF WHITE BLOOD CELLS OF HUMAN BLOOD GROUPED ACCORDING TO THE VALUE OF THE TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL LIPID ANALYSIS

(The lipid values are expressed in mg. per 100 gm. of white blood cells, moist weight)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos-pholipid	Free cholesterol	Cholesterol ester
500–799	21	Minimum	501	0	257	82	0
		Maximum	798	244	552	220	139
		Mean	667	68	433	146	20
		Standard deviation	89	73	92	31	48
		Standard deviation in per cent of mean	13%	107%	21%	21%	240%
800–1099	32	Minimum	800	0	313	22	0
		Maximum	1081	488	780	259	468
		Mean	985	180	550	160	95
		Standard deviation	95	144	117	53	103
		Standard deviation in per cent of mean	10%	80%	21%	33%	108%
1100–1399	26	Minimum	1105	0	440	72	0
		Maximum	1392	642	1095	375	591
		Mean	1250	216	681	208	145
		Standard deviation	117	184	185	63	164
		Standard deviation in per cent of mean	9%	85	27	30%	113%
1400–1699	27	Minimum	1403	0	627	117	0
		Maximum	1679	638	1375	393	316
		Mean	1537	165	1030	257	85
		Standard deviation	105	207	195	64	107
		Standard deviation in per cent of mean	7%	125%	19%	25%	126%
1700–1999	21	Minimum	1726	0	269	63	0
		Maximum	1966	1004	1550	452	917
		Mean	1900	361	1030	271	238
		Standard deviation	95	308	300	98	232
		Standard deviation in per cent of mean	5%	85%	29%	36%	98%
2000–2399	22	Minimum	2015	0	411	129	0
		Maximum	2353	1390	1770	472	943
		Mean	2174	527	1218	302	127
		Standard deviation	118	330	323	84	226
		Standard deviation in per cent of mean	5%	63%	27%	28%	178%
2400–2799	11	Minimum	2423	0	823	245	0
		Maximum	2782	1460	2410	553	912
		Mean	2591	442	1527	399	223
		Standard deviation	128	476	432	109	291
		Standard deviation in per cent of mean	5%	108%	28%	27%	131%
Over 2800	14	Minimum	2586	0	354	171	0
		Maximum	3951	2650	2770	680	818
		Mean	3360	1156	1586	385	233
		Standard deviation	287	943	680	147	252
		Standard deviation in per cent of mean	9%	82%	43%	38%	108%

the total fatty acid. A summation of experimental errors must thus appear in the values of both neutral fat and cholesterol ester which may in a large part account for their variability.

In Fig. 5 the mean values of the component lipids have been plotted against the total lipid. Phospholipid and free cholesterol may be seen to bear a direct linear relation to total lipid as in the red blood cells, although the actual values of both lipids were much higher in the white than in the red blood cells. Considerable significance has been attached to the concentration of phospholipid in the white blood cells. The phospholipid content of a tissue appears to vary with its physiological activity, or tone, or functional capacity, or degree of training in the gymnastic sense. In conformity with this general hypothesis, Boyd (8, 13) has shown that increased values for phospholipid are found in the white blood cells of patients recovering normally from infection, while in patients who succumb to infection or combat it weakly, the phospholipid content of the white blood cells falls or remains low.

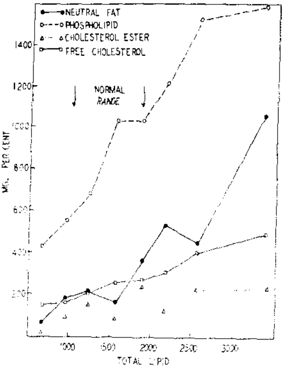

FIG. 5. *The relation of mean values of lipids in the white blood cells of healthy and diseased persons to mean values of total lipid expressed in mg. per 100 gm. moist weight.*

The increased amounts of neutral fat and cholesterol ester indicated by the rising mean curves in Fig. 5 cannot be taken as evidence that the values of these lipids bear a direct relation to the concentration of the total lipid. In Table VII it was shown that the standard deviation of the means for these lipids was relatively high. Hence it may be concluded only that frequently high values for cholesterol ester and for neutral fat appear when the total lipid content rises above the normal range.

The Interrelation of Lipids of the White Blood Cells in Normal Cases

The variations encountered amongst the lipid values of the leucocytes from all types of cases were greater than those seen amongst the normal cases as listed in Table VIII. The standard deviation of the total lipid values for normal cases extended from 4 to 14% of the means, of neutral fat values from 46 to 126% of the mean, of phospholipid values from 11 to 22% of the means, of free cholesterol values from 19 to 44% of the means and of cholesterol ester values from 54 to 144% of the means respectively. It is evident from Fig. 6 that the behavior of normal values for component lipids within the normal range of total lipid was analogous to that of all cases presented in Fig. 5.

TABLE VIII

THE LIPID COMPOSITION OF 30 SAMPLES OF WHITE BLOOD CELLS OF NORMAL HUMAN ADULTS GROUPED ACCORDING TO THE VALUE OF TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS

(The lipid values are expressed in mg. per 100 gm. of white blood cells, moist weight)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos-pholipid	Free cholesterol	Cholesterol ester
To 1099	8	Minimum	688	38	442	76	0
		Maximum	1076	397	630	242	306
		Mean	935	118	578	162	77
		Standard deviation	130	105	62	50	111
		Standard deviation in per cent of mean	14%	89%	11%	31%	144%
1100–1399	8	Minimum	1128	0	457	72	14
		Maximum	1385	346	920	489	237
		Mean	1250	173	704	265	108
		Standard deviation	117	138	152	63	98
		Standard deviation in per cent of mean	9%	80%	22%	23%	91%
1400–1899	9	Minimum	1522	0	860	277	0
		Maximum	1894	595	1290	452	280
		Mean	1740	164	1111	326	139
		Standard deviation	142	206	125	142	108
		Standard deviation in per cent of mean	8%	126%	11%	44%	78%
Over 1900	5	Minimum	1963	61	990	245	0
		Maximum	2142	668	1380	420	155
		Mean	2040	486	1112	327	115
		Standard deviation	74	223	146	61	62
		Standard deviation in per cent of mean	4%	46%	13%	19%	54%

The Normal Range of Lipids in the White Blood Cells

A composite statistical presentation of results for leucocytic lipids of normal human adults has been given in Table IX. The total lipid content of normal human white blood cells was calculated to have an expected range including 95% of cases from 603 to 2291 mg. per cent or approximately three times as great as occurs in plasma and the red blood cells. On the average the total lipid of the white blood cells was composed of 60% of phospholipid, 16.5% of free cholesterol, 14.5% of neutral fat and 9% of cholesterol esters. It is apparent from Table IX that single values for leucocytic neutral fat may be

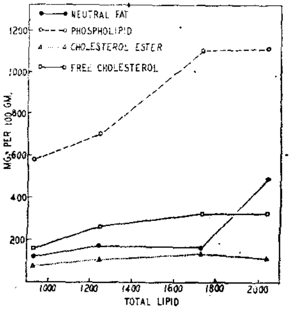

FIG. 6. *The relation of mean values of lipids in the white blood cells of normal healthy human adults to mean values of total lipid expressed in mg. per 100 gm. moist weight.*

TABLE IX

THE LIPID COMPOSITION OF THE WHITE BLOOD CELLS IN 30 COMPLETE DIFFERENTIAL ANALYSES ON NORMAL HUMAN ADULTS

(The results are expressed in mg. per 100 gm. of white blood cells, moist weight)

Value	Total lipid	Neutral fat	Phospholipid	Free cholesterol	Cholesterol ester
Minimum values*	688	0	442	72	0
Maximum values*	2142	668	1380	452	306
Mean values	1447	209	867	238	131
Standard deviation	422	212	281	95	121
Standard deviation in per cent of mean, %	29	101	32	40	92
Expected range of 2/3 of normal subjects	1025–1869	0–421	586–1148	143–333	10–252
Expected range of 95% of normal subjects	603–2291	0–633	305–1429	48–428	0–373

The sum of the individual lipids does not equal the total lipid because the individual lipid values are from different analyses.

considered normal if they occur within the range of from 0 to 633 mg. per cent. The corresponding expected range of 95% of values for phospholipid was from 307 to 1431 mg. per cent, for free cholesterol from 48 to 428 mg. per cent and for cholesterol ester from 0 to 363 mg. per cent.

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STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

V. FURTHER PARASITES FROM THE OCELOT[1]

By Thomas W. M. Cameron[2]

Abstract

Several nematodes are recorded from the Ocelot *(Felis pardalis)*; and the whipworm, *Trichocephalus serratus*, is described in detail.

In the third paper of this series, the parasites of various carnivores including an ocelot (*Felis pardalis*), were described. Recently the entrails of a second ocelot from Trinidad were sent by Professor F. W. Urich to the Institute of Parasitology. The animal proved to contain one species of Acanthocephala and three species of nematodes, all in small numbers.

Echinopardalis pardalis (Westrumb, 1821) Travassos, 1918

One male and two females were recovered from the small intestine. The male measured 7.5 mm. and the females 8.5 mm. and 9.5 mm. respectively. The testes are elongated and situated one behind the other in the middle third of the body. The prostate glands are posterior to the second testis and are situated in pairs, although not so regularly as is described by Travassos (4). A very short neck is also present. These slight differences from Travassos' description however do not seem to be of specific importance, especially as the specimens are obviously young ones.

This acanthocephalid apparently is essentially a South American form and has been recorded from a number of Felidae (including the present host) on that continent. It has also been found in the domestic cat in North America.

The ocelot also contained a few examples of *Ancylostoma caninum* (Ercolani, 1859) and of *Molineus pardalis* Cameron, 1936, as well as several whipworms.

Trichocephalus serratus von Linstow, 1879

Two male, one immature and three mature female whipworms were found.

Both sexes are remarkable for their very small size and for the great length of the "neck". The mature females varied from 15.75 to 13.5 mm. in total length (av. 14.75) while the males were 12.5 and 13 mm. respectively. The average body length of the female was about 2.75 mm. and of the male, 3.4 mm. This gives a ratio of 5 : 1 for the length of "neck" to the body in the female and over 3 : 1 in the male. The females are 0.35 mm. wide and the males 0.3 mm. The head end in each sex is similar and typical. There

[1] *Manuscript received September 16, 1936.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Quebec, with financial assistance from the National Research Council of Canada.

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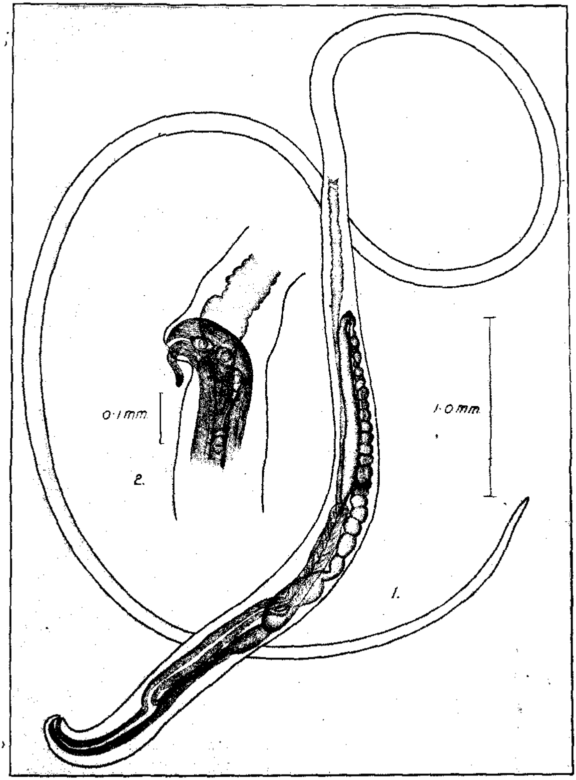
is a single broad, bacillary band present in the "neck", originating close to the head and terminating about the level of the body. The remaining part of the "neck" and all the body is finely striated transversely.

The female is a more or less typical whipworm, the vulva being situated at the junction of "neck" and "body". From the lower lip of the vulva



Trichocephalus serratus.

FIG. 1. *Entire male whipworm.* FIG. 2. *Vulvar region of female.*

however (Fig. 2) there projects a smooth finger-like process. The female genital system is typical. The ovarian tubule originates close to the anus and passing forward to the level of the vulva, turns back again to just anterior to its origin. There it turns and becomes a voluminous uterus which opens at the vulva. In the young specimen, this uterus appeared to be divided into two parts, but no such division was noticeable in the adults. In this same specimen, there were two pairs of minute sessile papillae just anterior to the anus. The eggs are of the typical whipworm type, show little variation in size, and measure (*in utero*) 60μ × 35μ.

The male (Fig. 1) has no terminal spiral and superficially resembles the female. Unfortunately in both cases the spicule was retracted but both specimens were well preserved and the details clearly visible. However, it is not possible to say whether or not the "sheath" is campanulate when extruded. It is spinose, and when retracted is about 0.4 mm. in length. The spicule, 1.2 mm. long, is simple and filiform. Its anterior end is attached to a large retractor muscle. About half way down the spicular canal, it is joined by a voluminous muscular tube which splits at its anterior end to join the rectum and male duct. This cloacal tube is presumably contracted towards the rear end of the body when the "sheath" is extruded. The male genital opening is dorsal to the rectum and a short canal joins the cloaca with a massive cement gland. This in turn communicates with the seminal vesicle by a narrow opening. The seminal vesicle runs as far as the junction of intestine and oesophagus where it communicates with the single testicle. This is a wide, laterally convoluted tube, which originates just posterior to the junction of the male and digestive tubes.

The digestive system is quite typical.

Whipworms are somewhat rare in Felidae and such records as exist all refer to South American cats. The sole reference to whipworms from this particular host is one by Canavan (2) who found a single female 42 mm. long in an ocelot which had died after a short period of captivity in the United States.

In 1851, Diesing recorded *T. felis* from *Felis tigrina* in Brazil but his species is not recognizable.

In 1879, von Linstow described *Trichocephalus serratus* from the domestic cat, and in 1889 he described *T. campanulus* from the same host; both examples came from Brazil. In 1923, Urioste examined some specimens from the cat from the same country and concluded a single species existed: this he called *Trichocephalus serratus*.

There are few details in this genus available for a morphological comparison of species and resource has to be made to the very unsatisfactory criterion of measurements.

	T. serratus (von Linstow)	*T. campanulus* (von Linstow)	*T. serratus* (Urioste)	Present species
Male	40.0 mm.	Not stated	20.5 mm.	12.75 mm.
Spicule	3.9 mm.	Not stated	1.5 mm.	1.2 mm.
Female	48.0 mm.	31.5 mm.	23.0 mm.	14.75 mm.
Eggs	56 × 39μ	72 × 36μ	80 × 36μ	60 × 35μ

T. serratus is stated to have a vaginal prolapse while in *T. campanulus* the vulvar lips are slightly salient; the projecting lower vulvar lip in the present species is no doubt the same structure interpreted by von Linstow as a prolapse. The differences between the present species and *T. serratus* are accordingly mainly differences in dimensions, those for body and spicular lengths of the present specimens being roughly one-third of *T. serratus*; the eggs however are almost identical in size. There seems no reason accordingly, why it should not be referred to von Linstow's first species. While the present evidence is too scanty to allow of a definite opinion, it seems probable that only a single species exists in Felidae. It is peculiar however that all records of whipworms in cats should be South American.

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Canadian Journal of Research

Issued by The National Research Council of Canada

VOL. 15, SEC. D. FEBRUARY, 1937 NUMBER 2

BIOLOGICAL NOTES ON THE CHRYSOPIDAE[1]

By Wm. L. Putman[2]

Abstract

This paper presents further biological and ecological data on the Chrysopidae gathered during a study of the predators of the oriental fruit moth. The species concerned are *Chrysopa rufilabris* Burm.; *C. plorabunda* Fitch; *C. downesi* R. C. Smith; *C. oculata* Say; *C. nigricornis* Burm.; *Meleoma signoretti* Fitch and *M. emuncta* Fitch. Data on life history include relation of development to temperature, number of generations, method of overwintering, and oviposition. The early stages of *C. downesi*, *M. signoretti* and *M. emuncta* are described. Seasonal prevalence and fluctuations in abundance from year to year are discussed and notes are given on natural control agencies. The value of chrysopids in the biological control of the fruit moth is summarized, with the conclusion that only in exceptional seasons are they of appreciable importance.

In a previous paper (1) the writer gave some account of the value of chrysopids as predators of the oriental fruit moth (*Grapholitha molesta* Busck), together with some notes on their habits and life histories. This paper presents further details of studies in which most attention was given to *Chrysopa rufilabris* Burm., *C. plorabunda* Fitch, and *Meleoma signoretti* Fitch, these being the most abundant and the only species of appreciable importance as predators of the fruit moth. More or less incidental notes were also made on *C. oculata* Say, *C. downesi* Smith, *C. nigricornis* Burm., and *M. emuncta* (Fitch). There is some doubt concerning the identity of the species here called *Chrysopa downesi* which is discussed on a later page.

Life History

All the life-history studies were carried on in the screened insectaries at St. Davids and Vineland Station.

Incubation period. It was possible to determine the incubation period of only a small number of eggs as very few were laid in captivity. The length of the period, with the mean daily temperature determined from thermograph records is given in Table I.

TABLE I

Incubation period of Chrysopids

Species	No. of eggs	Length of period, days	Mean daily temperature, °F.
C. rufilabris	26	6 – 7	68.6 – 64.8
C. plorabunda	12	5 – 6	73.3 – 66.6
M. signoretti	5	9	66.1

Larval period. The larvae were reared in glass shell vials plugged with cotton. They were supplied daily with fresh eggs or larvae of the fruit moth

[1] *Manuscript received December 29, 1936.*
Contribution from the Entomological Branch, Dominion Department of Agriculture.
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or Mediterranean flour moth (*Ephestia kuehniella* Zell.). The duration of the three larval instars was recorded for a few larvae and appeared to be approximately equal. The length of the complete period in relation to the mean daily temperature is given in Table II.

TABLE II

LARVAL PERIOD OF CHRYSOPIDS

Mean daily temperature, °F.	No. of larvae	Larval period, days	
		Extremes	Average
Chrysopa rufilabris Burm.			
77 - 77.9	3	10	10.0
76 - 76.9	4	9 - 13	10.8
75 - 75.9	26	9 - 12	10.0
74 - 74.9	52	10 - 14	11.5
73 - 73.9	43	9 - 14	11.1
72 - 72.9	35	10 - 15	11.9
71 - 71.9	49	11 - 15	12.6
70 - 70.9	45	11 - 15	12.7
69 - 69.9	18	11 - 15	13.0
68 - 68.9	15	12 - 15	13.3
67 - 67.9	8	12 - 14	12.8
66 - 66.9	1	14	14.0
65 - 65.9	9	14 - 18	15.8
63 - 63.9	3	16 - 18	16.7
62 - 62.9	1	17	17.0
59 - 59.9	2	24	24.0
Chrysopa plorabunda Fitch			
77 - 77.9	1	8	8.0
76 - 76.9	1	9	9.0
74 - 74.9	8	10 - 12	10.5
Chrysopa plorabunda Fitch—*Concluded*			
73 - 73.9	2	10	10.0
71 - 71.9	4	10 - 15	12.5
70 - 70.9	11	10 - 13	11.5
69 - 69.9	10	12 - 13	12.4
68 - 68.9	10	11 - 14	13.2
67 - 67.9	2	12 - 15	13.5
66 - 66.9	1	15	15.0
65 - 65.9	5	16	16.0
64 - 64.9	5	16 - 18	17.0
63 - 63.9	2	17	17.0
61 - 61.9	4	18 - 20	19.5
60 - 60.9	4	19	19.0
59 - 59.9	1	23	23.0
Meleoma signoretti Fitch			
73 - 73.9	1	15	15.0
71 - 71.9	4	15 - 19	16.7
70 - 70.9	3	15 - 18	16.0
69 - 69.9	8	17 - 18	17.6
68 - 68.9	15	17 - 19	18.5
67 - 67.9	2	18 - 19	18.5

Pupal period. The cocoons were left in the vials in which they were spun. There was considerable mortality in the cocoons, ranging from 13 to 37% based on 41 and 187 individuals respectively. Moisture was supplied by wetting the plugs of the vials occasionally. The period as given in Table III includes the prepupal stage, pupation occurring from three to six or more days after the cocoon was spun.

TABLE III

PUPAL PERIOD OF CHRYSOPIDS

Mean daily temperature, °F.	No. of pupae	Pupal period, days	
		Extremes	Average
Chrysopa rufilabris Burm.			
77 - 77.9	3	10 - 11	10.3
76 - 76.9	7	9 - 13	10.9
75 - 75.9	5	10 - 12	11.5
74 - 74.9	13	11 - 13	11.4
73 - 73.9	29	11 - 14	11.7
72 - 72.9	20	11 - 16	13.0
71 - 71.9	27	11 - 15	14.0
70 - 70.9	21	13 - 16	14.4
69 - 69.9	1	15	15.0
68 - 68.9	10	13 - 17	15.6
67 - 67.9	12	16 - 18	16.6
66 - 66.9	2	16 - 17	16.5
65 - 65.9	8	17 - 20	19.0
64 - 64.9	9	18 - 21	19.3
Chrysopa plorabunda Fitch			
74 - 74.9	2	10 - 11	10.5
73 - 73.9	1	10	10.0
71 - 71.9	1	11	11.0
70 - 70.9	6	11 - 14	12.2
69 - 69.9	2	13	13.0
68 - 68.9	2	13 - 14	13.5
67 - 67.9	4	12 - 14	13.3
65 - 65.9	18	14 - 19	18.0
64 - 64.9	1	19	19.0
61 - 61.9	2	20 - 21	20.5
60 - 60.9	1	19	19.0

Oviposition and longevity of adults. It was very difficult to get adults of *C. rufilabris* emerging in the insectary to oviposit, although cages of various types were used and the insects supplied with water and aphids, sugar solution, and yeast. A few eggs were obtained from cages placed on aphid-infested trees but the number normally laid is not known. When supplied with aphids, adults of this species lived as long as 45 days, but they died quickly when given water only.

C. plorabunda was much more easily handled in captivity and usually oviposited when caged on aphid-infested twigs. Adults of the summer generations deposited from 18 to 114 eggs and lived as long as 42 days. Over-wintering adults laid more than 56 eggs over a period of five weeks. An examination of the undeveloped ovaries of this species showed a potential production of at least 144 eggs.

Seasonal life history. *C. rufilabris* overwintered in the prepupal stage within the cocoons. These are spun among the leaves, with which they fall, on the twigs, and in considerable numbers inside the curls of the thin outer bark. The exact dates of spring emergence were not determined but they appeared to be early in June, as eggs were found as early as June 10. There appeared to be two complete generations and probably a partial third, but these were not distinguishable in the field because of the long oviposition period and the great variation in the length of the larval stages under conditions of food scarcity. The number of eggs present in the peach orchards usually increased rapidly from about the middle of June and reached a maximum about the middle of July, after which oviposition decreased rapidly. Eggs were very scarce after early August, although a few could be found till nearly the middle of September. All the eggs found up to and including the time of maximum deposition were laid by the spring brood of adults, and while in some years, particularly in 1933, there was some indication of a slight increase in the egg population in early August, the number laid by the first brood adults was very small and much less than that of the spring brood. The presence of the first brood in considerable numbers was shown by the bait pail catches, so it appeared that the decline in egg population was due to the reduced fecundity of the females of this brood. In the writer's previous paper it was conjectured that lack of food for the larvae was responsible, but an equally probable cause was insufficient nourishment of the adults. The experiments in connection with oviposition outlined above appear to show that aphids are necessary for the proper development of the ovaries. Green peach aphids, *Myzus persicae* Sulz., were usually common in the orchards during the greater part of June and a few usually remained until July, while the spring brood of *rufilabris* was active, but all migrated to other hosts before the appearance of first brood adults.

C. plorabunda hibernated in the imaginal state. Several were successfully wintered on the earth floor of the insectary under an inverted flower-pot. While none were actually discovered in midwinter, they were found in late

fall and early spring among dead grass and fallen leaves, in strawberry beds and in outbuildings. On warm days they were often active until late November and were seen in flight as early as March 22. On the approach of cold weather, in late October and November, the usual green color of the species changed to yellow mottled with brown, but reappeared early in May after they emerged from hibernation and began to feed. Both sexes hibernated, the females with undeveloped ovaries, and fertilization took place in the spring. In cages, oviposition commenced about the first of June and continued nearly to July. In the orchards, eggs were not found before June 27 and in some years not until nearly a month later. Apparently the adults did not hibernate in the orchards because of lack of shelter, and did not invade them in noticeable numbers until the time of the late spring brood or early first brood oviposition. After this time eggs could be found during most of the summer, sometimes till September 11, but there were no consistent periods of maximum oviposition and the broods could not be distinguished. As this species produces eggs earlier in the season and the developmental period is somewhat shorter, three complete generations are possible, but it is doubtful whether the third is very large.

Meleoma signoretti passed the winter as a prepupa within the cocoon. Adults emerged between June 2 and 5 from a few cocoons overwintered in the insectary. In 1932, the only year in which any appreciable numbers were present, the first eggs were found in the orchards on July 5, the maximum number on July 20 and the last on August 24. There was one complete generation and, at least in some years, a partial second. In 1931, first brood adults were taken in the bait pails from August 18 to September 8, while in 1932, when the species was much more abundant, none were taken, and larvae maturing as early as August 7 did not emerge as adults that season.

Food Habits of the Larvae

Larvae of *C. rufilabris* were seen in the orchards attacking eggs and larvae of the oriental fruit moth, nymphs of the cottony peach scale (*Pulvinaria amygdali* Ckll.), European fruit lecanium (*Eulecanium corni* Bouché), white fly (*Trialeurodes vaporariorum* Westw.), the leaf-hopper, *Erythroneura obliqua* Say, and all stages of the European red mite (*Paratetranychus pilosus* C. & F.). They would apparently destroy almost any soft-bodied insect not too large or active to handle, but could not be reared satisfactorily on green apple aphids (*Aphis pomi* DeGeer) and also refused the woolly species of aphids.

There was less opportunity for observing the food preferences of *C. plorabunda* and *M. signoretti*, but these species and *C. downesi* ate fruit-moth and flour-moth eggs and larvae and aphids in the insectary and were apparently similar to *rufilabris* in their choice. *C. oculata* appeared to be chiefly an aphid feeder, as only a few larvae could be induced to accept fruit-moth eggs and then only when starved. The habits of *C. nigricornis* appeared to be very similar.

Fluctuations in Abundance

As part of an ecological study of the oriental fruit moth, population studies of chrysopid eggs were made from 1931 to 1935 at four selected orchards in the Niagara Peninsula. These were located at St. Davids, Niagara-on-the-Lake, Vineland Station, and Grimsby Beach, covering about 22 miles of the fruit belt. Briefly, the procedure was to make one-hour counts at chosen points in the mature orchard at weekly intervals from approximately June 1 to September 10. Duplicate counts were also made on young trees at each point, but because of the variation in the age of the trees and the necessity of choosing a new block as the original one became too old, it was thought that the figures were not comparable and were discarded. Approximately a thousand leaves were carefully examined at each hourly count and all fruit-moth eggs and other insects also recorded. The chrysopid eggs were collected and reared to determine the species. In Table IV the combined counts for each season are given.

TABLE IV

CHRYSOPID EGG POPULATION IN PEACH ORCHARDS

	1931	1932	1933	1934	1935
C. rufilabris	508	11	83	262	119
C. plorabunda	90	19	5	16	19
C. nigricornis	—	32*	—	—	—
C. oculata	—	6	18	68	19
M. signoretti	—	34	3	—	1

* *1 cluster of 32 eggs.*

Unfortunately no counts were made in 1930 when chrysopids were more abundant than in any succeeding year. A perusal of the table will show a great variation in both the abundance of each species during the five-year period and the relative numbers of the species in each year. *C. rufilabris* was by far the most prevalent species in every year except 1932, when it was relatively scarce; it occurred in greatest abundance in 1931 and was again very common in 1934. *C. plorabunda* was always much less abundant except during 1932, and was also most prevalent in 1931. *M. signoretti*, usually an uncommon species, suddenly became quite common in 1932, but very few were found during succeeding years. The few eggs of *C. oculata* were collected largely during June, while aphids were still present on the trees, and it should be noted that the greatest numbers were found in 1934, a year of unusually heavy aphid infestation. Eggs of *C. nigricornis* were found on only one occasion.

The larvae of *rufilabris*, *plorabunda* and *signoretti* were commonly observed on the trees in numbers roughly proportionate to the egg populations. Exceedingly few *oculata* were found, the larvae apparently migrating to the soil or low vegetation. One or two larvae of *C. downesi* were collected and a few eggs found in another orchard.

Natural Control Factors

Infertility of eggs. Approximately 5 to 9% of the eggs collected in the orchards failed to hatch. These figures were derived from collections of 103 and 180 eggs respectively. Some of this mortality might have been due to injury in collecting, but many eggs appeared to be infertile.

Egg parasitism. Parasitism of the eggs by *Trichogramma minutum* Riley ranged from traces in 1933 and 1934 to 5% of counts of 290 and 179 eggs in 1932 and 1935 respectively, and 7% of 987 eggs in 1931. In individual orchards it reached 12% of 221 eggs examined in 1931 and 50 examined in 1932. Chrysopid eggs in years of abundance may be of some importance as a reservoir in which a stock of *Trichogramma* is built up to attack the later generations of the fruit moth.

Food supply of larvae. Lack of sufficient food appeared to be one of the most important factors limiting the chrysopid population. In some years chrysopid eggs were often the most prevalent form of life on the trees and under such conditions very few larvae could survive.

Pupal parasites. These are not properly pupal parasites as they attack the prepupa. The only primary parasite discovered was *Chrysopoctonus rileyi* Ashm. which was reared several times from summer generation cocoons of *rufilabris*, *plorabunda*, and *nigricornis* and probably attacks all species. The most abundant species was *Hemiteles tenellus* Say, reared many times from cocoons of both summer and overwintering generations of *rufilabris* and *plorabunda*. An examination of the contents of the cocoons proved that this species was hyperparasitic, probably on *Chrysopoctonus*, and in view of its abundance must be a decided check on parasitism by the latter species. One specimen of another hyperparasite, *Perilampus chrysopae* Cwfd., was obtained, and a few undetermined chalcids emerged from another cocoon. It was impossible to determine the extent of parasitism at any one time because of the scarcity of cocoons, but a lot of 31 old cocoons collected in 1932 from peach trunks, and representing all generations for two years or more showed a parasitism of approximately 50%.

Destruction of cocoons by predators. Some of the cocoons found on the trunks had been torn open by some predator and the pupae devoured. Some coleopterous larvae resembling *Tenebroides* were found in the same location and may have been responsible.

Natural mortality within the cocoon. Many cocoons were found containing dead pupae or prepupae. Among those collected on the trunks this natural mortality reached approximately 26%.

Food supply of adults. The possibility that a deficient food supply may limit the fecundity of the females has been discussed on a previous page.

Summary. The most important factors limiting the chrysopid population are lack of food for the larvae and possibly for the adults, and parasitism; but the manner in which these and probably other unknown influences, such as weather conditions, cause the great fluctuations in numbers is not known.

Descriptions of Immature Stages

The early stages of most of the species concerned have been well described and figured by Smith (2) and only those not previously known are described here.

Chrysopa downesi SMITH

There may be some doubt concerning the identity of the species here called *downesi*. A small series was identified as this species by its author, Dr. R. C. Smith, but none of the hundred or more examined by the present writer have the black band over the genae mentioned in the original description (3), but have a red band only, in this respect agreeing with *harrisii* Fitch. There is some variation in size and the relative widths of the wings but only one species appears to be represented. The adults in life have a characteristic bright, deep green color which soon disappears in mounted specimens.

Egg. Stalked; yellowish-green; indistinguishable from that of *plorabunda*.

Larva. Unfortunately no detailed description of this species was made, but the only noticeable distinctions between it and that of *plorabunda* were the markings of the head. These consisted of two longitudinal bands converging somewhat toward the base of the head, as in *plorabunda*, but of uniform width throughout and not expanding at the base as in the latter species.

Cocoon. Oblong-spherical, of pale yellow-green silk. Cocoons of other species may appear greenish but this is due to the enclosed pupa. Length 3.2 mm.; width 2.8 mm.

Meleoma signoretti FITCH

Egg. Of typical chrysopid type; stalked; deposited singly.

First instar larva. General color pale. Head with two conspicuous longitudinal, somewhat reniform black marks, concave toward the middle line, very similar to the second instar. Smith's figure ((2), Plate LXXIV) doubtfully referred to this species, is apparently correct. Thoracic segments each with two large dark spots. Abdomen with obscure darker markings. Tubercles with long, curved pale setae. Legs pale, apices of segments ringed with dusky. Width of head, 0.39 mm.; length of mandibles, 0.39 mm.; antennae, 0.57 mm.; longest setae, 0.52 mm.; total length, 2.07 mm.

Second instar larva. (Fig. 1, a). Head similar to first instar but spots more angular; jaws pale amber, darker apically; antennae and palpi pale brown. Thorax pale yellowish; prothorax with two dark longitndinal im-

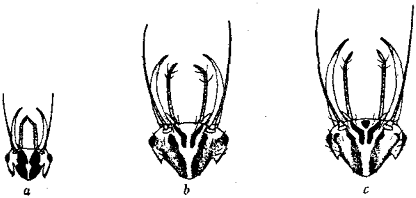

FIG. 1. *a. Meleoma signoretti; second instar larva. b. M. signoretti; third instar larva. c. M. emuncta; third instar larva.*

pressions (sclerites) and marked with pale reddish; meso- and metathorax each with two brown marks on either side, the posterior larger and extending diagonally outward and backward to small black impressions; pale reddish spots external to brown ones; dorsal vessel brown with lighter brown irregular stripes on either side; tubercles pale marked with reddish. First abdominal segment without tubercles; those of second segment marked with brown. Abdomen pale yellow, with irregular broken brown markings tending to form two bands laterally which become darker and converge toward the caudal extremity; brown markings interspersed with pale reddish. Thoracic and abdominal tubercles well developed, with curved pale setae. Legs pale. Width of head, 0.74 mm.; length of mandibles, 0.59 mm.; antennae, 0.98 mm.; longest setae, 0.50 mm.; total length, about 5 mm.

Third instar larva. (Fig. 1, b.) Head with two pairs of curved black marks, the inner on the anterior part of the head, extending forward, diverging and angled outward to the base of the jaws; outer pair shorter and broader, not extending as far anteriorly and merging into broad pale brown bands extending posteriorly to the base of the head; from the outer side of these, similar broad bands extend diagonally outward and forward to the eyes; a dark band extending along side of head from base nearly to eyes; eyes black. Jaws amber brown, darker apically. Antennae and palpi pale brown. Thorax and abdomen similar to second instar but markings paler and more diffuse; black impressions on prothorax divided longitudinally and a central black spot above dorsal vessel. Width of head, 1.04 mm.; length of mandibles, 0.95 mm.; antennae, 1.45 mm.; longest setae, 0.75 mm.; total length, approximately 10 mm.

Cocoon. Somewhat oblong spherical; white; loose outer layer sometimes with a reddish tint when new; length 3.8 mm.; width 3.3 mm.

Meleoma emuncta (FITCH)

Egg. Indistinguishable from that of *M. signoretti;* laid singly.

First instar larva. Head with two large longitudinal black marks which meet along the middle line, leaving triangular light areas at base and apex of head; a narrow black line in the light areas on either side of the large marks extending from the base half the length of the head; regions about the eyes black; mandibles amber-colored. Thorax and abdomen pale reddish brown; lateral tubercles white except second abdominal which are dark; setae long and curved, pale. Legs pale.

Second instar larva. Head similar to first instar; ground color pale yellowish gray. Thorax pale yellowish; prothorax marked with pale reddish and with two longitudinal black impressions; mesothorax and metathorax marked with brown, tending to form two oblique bands converging posteriorly on each segment with a smaller spot external to them; sides of mesothorax below and behind lateral tubercles dark; tubercles pale, marked with pale reddish, large and prominent. Abdomen darker yellowish, finely marked with brown, more heavily and tending to form two bands toward the caudal extremity;

dorsal vessel pale grayish brown, bounded by paler lateral areas; sides of second abdominal segment, including lateral tubercles, dark brown to black; tubercles on other segments finely spotted with brown on bases; setae on all tubercles long, curved, partly black.

Third instar larva. (Fig. 1, c.) Head pale yellowish marked with dark brown or black; from centre of head two black bars extend parallel anteriorly and then diverge sharply toward bases of jaws; a median triangular black spot between the bands near the anterior margin; two short oblique bars external to central bands; from these bars paler brown bands extend forward to bases of antennae and backward to base of head where they become much broader and give off similar bands obliquely outward nearly to eyes, enlarged, darker, and frequently somewhat crescent-shaped at the apices. Mandibles amber-colored, darker apically. Antennae and palpi pale brown. Thorax and abdomen similar to second instar, but brown markings paler and interspersed with pale reddish; sides of mesothorax pale. Legs pale. Width of head, 1.14 mm.; length of mandibles, 1.00 mm.; antennae, 1.54 mm.; total length, about 10 mm.

Cocoon. Similar to that of *M. signoretti.* Length, 4.00 mm.; width, 3.3 mm.

The larvae of *Meleoma* are easily distinguished from those of *Chrysopa* by their yellow-brown color and by their head markings. *Emuncta* larvae can be separated from *signoretti* by the median triangular spot near the apex of the head in the former species.

Influence of Chrysopids on the Oriental Fruit Moth

The only species with such habits and occurring in such numbers that they are potential factors of importance in the control of the fruit moth are *Chrysopa rufilabris*, *C. plorabunda*, and *Meleoma signoretti.* There is little to add to the writer's account of their destruction of eggs given in his previous paper (1), but they have since been found occasionally attacking larvae in the field. Unfortunately we still have no method of giving a numerical value to their influence, but there is no doubt that in 1931, and still more so in 1930, chrysopids were of considerable importance in the control of the moth, but from 1932 to 1935 they were apparently of minor value. The period of greatest abundance coincides very closely with the time the second brood eggs are laid and the greatest check is exerted on this brood.

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STUDIES ON THE HETEROPHYID TREMATODE *APOPHALLUS VENUSTUS* (RANSOM, 1920) IN CANADA

PART II. LIFE HISTORY AND BIONOMICS[1]

By Thomas W. M. Cameron[2]

Abstract

The eggs of the trematode pass into water, embryonate and are swallowed by the snail, *Goniobasis livescens*. They do not hatch in water. In the snail, multiplication through redia and daughter redia stages takes place and cercariae, with long flanged tails and pigmented eyespots, are produced. These escape into the water, but have a free life of less than 48 hours. To survive during this period, they must penetrate the skin of a fish, a great variety of which are successful intermediaries. In the muscle of the fish, the cercaria encysts to become metacercaria, which is however not immediately infective. Infection of the definitive host is by ingestion of the uncooked fish.

When *Apophallus venustus* was found to be endemic in the lower Ottawa Valley, preparations were made to attempt to elucidate the life cycle. While the life history of all the species in this genus was quite unknown, it was known that, in such other members of the family as had been studied, two successive intermediaries were essential. The second of these was always a fish, and the first an operculate snail.

Accordingly, a large number of freshwater fish was obtained locally and fed to kittens which had been raised in the college and had never been fed on fish. With a very few exceptions, every species obtained was found to be infective. *Apophallus venustus* was obviously, like the other members of the family, carried by fish.

The search for the snail intermediary was more difficult. By analogy from what was known of the other members of this family, it should prove to be an operculate snail. Moreover, all the cercariae already known from the Heterophyidae have pigmented eye-spots and peculiar flanges on their tails. Finally, the high incidence of infection in the fish suggested that the snail vector would be common locally and that a large percentage would be infected.

Search was accordingly made in the first instance for common species of operculate snails in the Ottawa River. Five species were found and I wish to acknowledge the invaluable assistance of Mr. A. LaRocque, of the National Museum, Ottawa, in identifying these.

The commonest local operculate snail was *Amnicola limosporata* which was found in thousands on the stems of various water weeds. Several hundreds of these snails were crushed and examined microscopically. No trace of any kind of trematode larva was found.

[1] *Manuscript received November 3, 1936.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Quebec, with financial assistance from the National Research Council of Canada.

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Campeloma decisum, a large species living on decaying vegetable matter, was also collected in some numbers, although it was much less common. No cercariae were found in this snail either.

Bulimus (Bithynia) tentaculatus is also very common locally, although it is a species introduced from Europe. This made it an improbable intermediate host for an endemic American trematode; nevertheless large numbers were examined but all proved negative.

These three species were subjected to artificial infection without result.

A species of *Pleurocerca*, a St. Lawrence form occurring in the Ottawa River only at its point of junction with the St. Lawrence River at Lake St. Louis, was obtained in small numbers. None was infected.

The remaining species, *Goniobasis livescens*, was first collected from stones just off Lynch Island, two miles east of Ste. Anne de Bellevue. It was present in large numbers and an examination of the first batch of 82 snails showed that 31% were infected with a cercaria which was very similar to that described by other workers from various species of Heterophyid trematodes. It seemed probable that this was the larval stage of *Apophallus venustus*. No other species of Heterophyid has been found locally in wild or domestic mammals or birds, and no other heterophyid cercaria has been found in any other species of local snail, although large numbers of all the common species, both operculate and non-operculate, have been carefully examined by my colleagues and myself in the laboratory during the past three years in connection with this and other trematode life cycles.

Positive proof of the life history of a trematode depends upon the production of all stages in the laboratory, in animals raised under circumstances which absolutely preclude any accidental contamination. In this particular case, it postulates laboratory-bred cats, laboratory-bred fish and laboratory-bred snails. The first of these conditions was easily fulfilled and the cats used have definitely not been exposed to natural infection. Attempts to breed suitable fish (such as catfish and black bass) in the laboratory under conditions which would satisfy these postulates, were unsuccessful and this was likewise the case with *Goniobasis livescens*.

Early in 1935, a large number of very young catfish had been collected and kept in the aquarium at the Institute of Parasitology. A number of these died from one cause or another but no encysted cercaria could be found on compressing the whole fish in a trichina compressorium. Others were later subjected to infection with the cercariae emerging from the snails and the larval flukes observed actively penetrating the skin of the fish. Experimentally infected fish were killed and examined from time to time and the encysted cercariae in the flesh were observed to develop and require some time to become mature. Negative results followed the feeding of cats with catfish containing young cysts and it seems reasonably sure that no previous infection with this fluke was present in the fish. One of these fish, a month

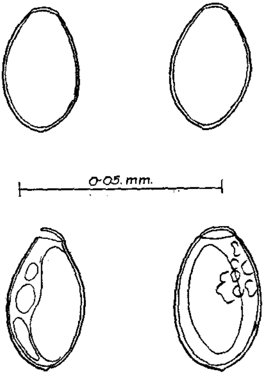
after its last exposure to infection, was fed to a kitten; three weeks later the cat was autopsied and *Apophallus venustus* found in its intestine. These experiments did not, however, furnish absolutely conclusive proof of identity.

A number of catfish was collected from an artificial pond on the grounds of Mr. J. J. Harpell, of the Garden City Press, Gardenvale, Quebec. These were young fish, bred in this pond which contained no operculate snails of any kind. They were retained in the laboratory for a month after experimental infection, and then fed to a cat. The result was positive. At the same time, a number of catfish from the same pond was collected and fed directly to another cat, without being exposed to the cercariae; the control was negative.

Thus the entire life cycle has not been observed in an entire experimental series. However, the high percentage of infected *Goniobasis livescens*, the presence of a single species of heterophyid cercaria in the area, and of a single species of adult heterophyid trematode in all the local animals examined, together with the artificial infection of fish and the large number of negative controls, is quite conclusive evidence that all the stages found belong to the same species of trematode.

Egg-miracidium Stages

The egg is more or less oval in contour but varies quite considerably in different specimens (Fig. 1). In a few it is distinctly constricted at the opercular end and the operculum is quite obvious; in others, it is an almost perfect oval and the operculum is seen with difficulty. Frequently there is a small thickening at the end opposite the operculum. In size it varies from 26μ to 32μ long and 18μ to 22μ wide, the mean size of a considerable number being 29μ by 20μ. In color it is light brown, and the outer surface has many honey-combed, irregular lines on it. When deposited in the intestine, it is not embryonated and I am unable to state the exact time necessary for the development of the miracidium. In the laboratory, in shallow water in Petri dishes, at least two weeks were required but further data on this are necessary.



Apophallus venustus
FIG. 1. *Outline drawings of four eggs showing variation in outline.*

The egg does not appear to hatch naturally, and specimens kept in the laboratory for almost a year did not hatch. As the pressure necessary to force off the operculum also invariably destroyed the miracidium, I have been

unable to ascertain details of this larval stage. It does not however, completely fill the egg shell, which also contains several large globules of a fat-like substance (Plate I, Fig. 2). Its general appearance is very similar to that of *Cryptocotyle* and *Opisthorchis*. The miracidium appears to hatch only after ingestion by the snail. Several snails, from a stock known to be parasite-free, were exposed to infection by placing them for a few hours in a Petri dish, the bottom of which was covered with washed feces containing eggs. The snails were then removed and placed in a clean aquarium jar and ultimately mother-redia were found in them. The snails unfortunately died at this stage, probably as the result of superinfection.

The culture of eggs was examined immediately after the removal of the snails and no hatched eggs were seen. This removes the possibility of some special stimulus, associated with the snail, being responsible for hatching, and leaves actual ingestion as the only alternative.

Snail Host (Plate I, Figs. 3, 4, 5)

As mentioned in the introduction, the only species of local snail found naturally infected was *Goniobasis livescens*. They are relatively abundant in parts of the Ottawa and St. Lawrence Rivers, near Ste. Anne de Bellevue, in areas where the necessary environmental conditions are found.

The genus has its centre of distribution in the southern United States, and LaRocque* is of the opinion that only two species occur in eastern Canada, *viz., G. haldemani* in Lakes Erie and Huron and *G. livescens* in the lakes and rivers of the drainage area of the Great Lakes and St. Lawrence—except those emptying into the St. Lawrence below the salt water line. All the specimens of *Goniobasis* used in the present work appear to belong to this species.

Goniobasis livescens has a thick, solid, more or less spiral, elongated shell, with a subrhomboidal aperture, closed by a subspiral horny operculum. There is a tendency among conchologists to divide this species into varieties which correspond with differing habitats. Its general habitat is a boulder bottom or on stones with sandy spots. Its presence in the latter area can usually be traced by the deep furrows which it makes as it moves along the sand. It is generally found in from one to three feet of water, in situations exposed to the river current, where it feeds on desmids and diatoms.

Baker (1) describes its behavior in an aquarium as follows:

"It will glide up the side of the glass jar with as much celerity as a *Lymnaea* or *Physa*, though not as rapidly. When at rest on the bottom, it moves more or less slowly, pushing its foot forward a short distance and drawing the shell after it, a sort of stepping process. While in motion, the rostrum is constantly in motion searching for food, the action of the radula being plainly

* *Museum Assistant, Division of Paleontology, Geological Survey, Department of Mines and Resources, Ottawa.*

seen as it scrapes the stones and other objects. The mouth may be opened and the radula seems to be used in a lapping manner. The long tentacles are constantly in motion, being used as tactile organs feeling and testing all objects within reach. Sight is apparently deficient, the tentacles performing the function of this sense."

Our experiences with this species are fully in accord with Baker's.

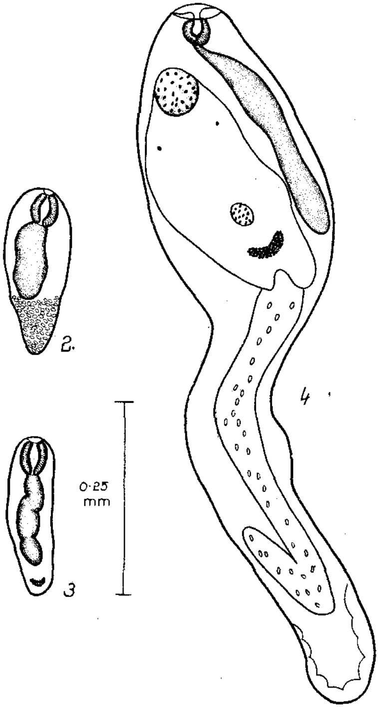
While *Goniobasis* occurs in a variety of places in the local waters, the most plentiful sources of supplies were found to be the Isles de la Paix on the St. Lawrence and the east end of Lynch Island, about two miles down the Ottawa River from Ste. Anne de Bellevue. Large numbers were collected from both these areas. All snails were examined for infection shortly after being brought into the laboratory and at intervals during the succeeding two months. The specimens that died were examined by crushing and searching under the binocular dissecting microscope. Living snails were placed individually in quarter-pint glass cream bottles, filled with water and examined with a hand lens for escaped cercariae 24, 48 and 72 hours later. Negative examples were placed in a stock jar, positive examples used as a supply of larvae. Negative examples were re-examined periodically and further segregated. In no case was any naturally infected snail found in the St. Lawrence islands. However, a large number of infected snails was found near Lynch Island in the Ottawa River; in one batch of 82 examples (part of a single day's collection) 26 infected snails were found—an infection rate of 31.6%. As such natural bird hosts as might exist or have been discovered are common to both areas, I can give no reason for this heavy infection rate or for the complete absence of any infection on a group of islands in the St. Lawrence, only four miles distant. In the spring of 1936, a large number of snails of this species was obtained from the Rideau River in Ottawa city. Two hundred of these were crushed and examined; two contained the cercariae of this trematode and two contained rediae and daughter rediae which may also belong to the same species, but which were too immature to identify.

Intra-molluscan Development

There has been little opportunity to examine the intra-molluscan development of the parasite and, as mentioned above, experimental infection was carried only as far as the mother-redia stage. The sporocyst stage has not been identified and all subsequent stages occur in the liver (Plate I, Fig. 6).

Young mother rediae were seen only in experimental infections, infected on October 30, 1935, and examined on November 16. At that stage of development, they are carrot-shaped organisms, about 0.22 mm. long (Fig. 2), with an almost spherical oral sucker at the blunt end, and a very large single caecum extending posteriorly for about two-thirds of the body length. The remainder of the body is filled with spherical cells. These rediae become much larger and are ultimately filled with daughter rediae (Fig. 3), which,

in their young stages, are small oval bodies with an oral sucker and a single caecum. The daughter rediae can move about by vermiform contractions of the body, but possess no hooks or spines. When fully mature (Fig. 4), they are elongated sausage-shaped bodies, with no appendages and apparently no functional birth pores. They contain a varying number of cercariae—from one to eight—rarely more (Plate I, Figs. 7, 8). These cercariae are fully developed morphologically before they leave the daughter rediae.



Apophallus venustus

FIGS. 2, 3 AND 4. FIG. 2. *Young mother redia.* FIG. 3. *Young d..ughter redia.* FIG. 4. *Mature daughter redia, containing a single cercaria. All drawn to same scale.*

Cercaria

The cercaria (Figs. 5, 6) is a very muscular larva and subject to great changes in body shape. This description is based on relaxed, living specimens, supplemented by sections and whole mounts of cercariae killed in formalin and stained with alum carmine. It has a long tail about one and one-half times the body length—sometimes a little longer, but never more than twice the body length. It is provided with a complete dorsal and an incomplete ventral fin of a very delicate structure (Plate II, Fig. 1). The dorsal fin originates as a very small fold close to the body and, gradually increasing in width, reaches its maximum about half-way down the tail; thereafter it becomes slowly reduced in size. It is continued around the ventral side to expand again to form a ventral fin, which however terminates about the mid-point of the tail. In a minority of specimens, there is a second ventral fin close to the body (shown as a dotted line in Fig. 5). The membranous fin is longer at its free edge than on its attached edge and it is, in consequence, undulated. This tail is very muscular and is attached, by a "ball and socket" mechanism to the posterior end of the body. It is very easily detached by its own rapid movement.

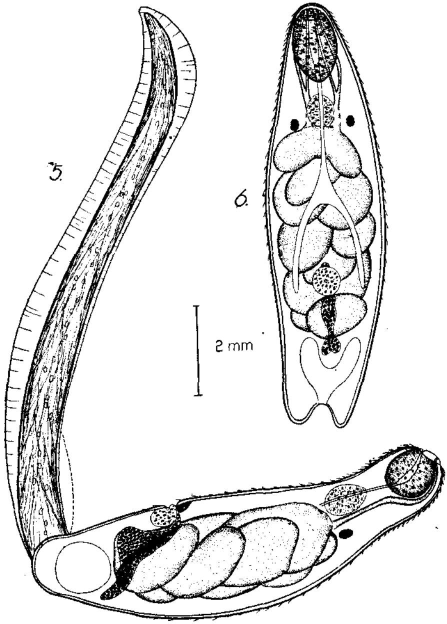
The body is about 0.10 to 0.13 mm. long and about one-third of this in width (Fig. 6). The oral sucker is spherical to oval in shape and only feebly muscular. It is about 20μ in diameter. It is sub-terminal in position and the mouth opening is towards the ventral surface. There are no hairs on the body. However, the whole surface of its anterior part to about its mid-point, is thickly covered with minute spines (as in the adult) except for a small area surrounding the termination of the oral sucker and just dorsal to it. The spines at the edge of this area are slightly larger than the rest. The spines on the posterior half of the body are few in number. The oral sucker is pierced by the oesophagus, which continues as a minute simple tube, to a point just in front of the middle of the body. There the tube bifurcates, and in one specimen the bifurcations were traced as far as the level of the ventral sucker. About mid-way between the bifurcation and the anterior end, the oesophagus is surrounded by an oval muscular pharynx.

Dorsal to the pharynx are two darkly pigmented eyespots of varying size.

The ventral sucker is rudimentary and appears to have no muscular fibres at this stage. It is however, provided with a small central depression. Just anterior to this is another small depression, surrounded by a mass of small cells—the rudiments of the genital sinus complex (Plate II, Fig. 4).

Passing from this point, dorsally and posteriorly, is a mass of cells, which stains deeply in preserved specimens and obviously represents the genital primordia. This mass (Plate II, Fig. 2) expands at about its mid-point and then commences to form two terminal swellings. Viewed from the side (Fig. 5) it is roughly "L" shaped. On the lateral margins of the body posterior to this are pigmented areas, and between these two areas lies the "Y" shaped excretory vesicle. I was unable to trace the excretory system in detail,

although a number of flame cells was observed in different parts of the body. Practically the whole of the centre of the body is filled with 16 large glandular cells which are readily demonstrated by vital staining. The ducts of these cells pass anteriorly in two medial bundles, which divide into four bundles of



Apophallus venustus
FIGS. 5 AND 6. FIG. 5. *Cercaria, side view.* FIG. 6. *Body of cercaria, from ventral aspect.*

four ducts each, just before the oral sucker is reached. These pass forward, in a dorso-lateral direction, to open on the spineless area just dorsal to the oral sucker.

This spineless area can be protruded slightly and this enables these glands to come into close contact with the skin of the fish and assist in penetration. Although there is no proof of the fact, it is probable that these glands secrete a histolytic ferment used in penetration. It is possible that a negative pressure from the oral sucker helps to maintain the larva in place during the time this

digestive process takes place; the ventral sucker appears to play no part in the action, at least in such specimens as were observed in the act under the microscope.

This cercaria, while possessing the same general characters as other members of this family, differs from them in detail. From the cercaria of *Cryptocotyle lingua* (which is the nearest relation of this species, the life cycle of which is known, and which develops in the marine gastropod, *Littorina littorea*), it differs in its shorter tail, smaller oral sucker, 16 instead of 18 glands, and in the presence of a rudimentary ventral sucker. (5)

The conditions of emergence of the cercariae are not yet fully understood. Temperatures within the limits prevailing naturally (18° C. to 24° C.) appear to have no effect. There was, however, a definite time influence which may be connected with light intensity. From a number of snails examined at hourly intervals during the day (10.00 a.m. to 12.00 midnight), it was found that none emerged before 4.00 p.m., a few between 5.00 and 8.00 p.m. and most between 8.00 and 10.00 p.m. Few emerged during the period between midnight and 10.00 a.m., and the optimum time appeared to be 8.00 to 9.00 p.m. These experiments were conducted at the beginning of October. All cercariae died within 48 hours of emergence in aquaria in the laboratory.

In jars in the aquarium, there is a definite tendency for the cercariae to congregate on the side next to the windows and towards the top of the water. They are most strongly attracted by direct light (on a southern window sill) and when the sun is obscured by a cloud they spread out and become less concentrated and less active. Artificial shadows have a similar effect. They are not only positively heliotropic, but are stimulated by light. This causes a reduction in their free life, and cercariae exposed in a north window do not live more than 24 hours, while those kept in complete darkness may survive for 48 hours.

They swim head foremost, with rapid figure-of-eight lateral lashings of the tail; this is done so rapidly that the entire larva becomes a blur. At the end of this rapid movement, the cercariae become suddenly quiescent and lie in the water with the body horizontal, the dorsal surface being downwards and the tail upwards at right angles to the body. In this position, they slowly sink in the water, suddenly to start rapidly swimming again. The swimming is generally vertically upwards and is generally carried out in short spurts of a few seconds each, the cercariae resting and falling a short distance between spurts. Only occasionally do they move in a diagonal direction.

In addition to moving through fluid by the action of their tails, the cercarial body is very muscular and it is able to crawl actively—especially after losing its tail—by worm-like contractions and expansions of the body.

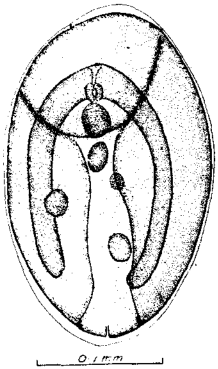
Contrary to Stunkard's experience with *Cryptocotyle lingua*, penetration of the fins by the cercaria can be observed with ease. Young catfish, about two inches long, are easily confined in a Petri dish of water which is placed on the stage of the binocular dissecting microscope. The cercariae, collected after emergence from the snail, swim about rapidly in the water for a short time. As soon, however, as one comes into contact with a fin—the body of the fish

appears to have little attraction for them—it holds itself against the skin by violent movements of its tail. These movements are so violent, that the tail is quickly detached. Meanwhile, the cercaria has been evidently digesting its way into the skin by means of the contents of its glands. It generally selects a position near the free edge of the fin, where the skin is thinnest, and within ten minutes it has entered. It generally succeeds in entering a blood vessel up which it moves by alternate contraction and expansion of its body, and it may be assumed that its spines are of considerable assistance in the process. Within 15 minutes of exposure to infection, the tail-less cercaria is seen disappearing into the body of the catfish and small drops of blood may be seen escaping from the open wound at the tip. This process has been repeatedly observed in these small fish. The young catfish are unable to stand the shock of too many penetrating cercariae and a number died from their effects. The bony dorsal and anal fins are the sites of election, although, less frequently, the tail fin also is attacked. Cercariae have never been seen attacking the fleshy dorsal fin of these fish, nor have metacercariae been found in the actual skin of any species of fish; they are always under it or in the muscles. The cercaria encysts in the musculature to become the metacercaria and it is further surrounded by an adventitious cyst formed by the fish itself.

The Metacercaria

The metacercaria (Plate II, Figs. 5, 6), after encystment in the fish, requires considerable time to become mature and infective. Experimental evidence is still lacking as to the exact time necessary, but it appears to be about four weeks. Once mature, probably it can live for a very long period—certainly over winter.

The mature metacercaria is virtually a young adult enclosed in a thin-walled, strong, elastic, hyaline cyst, which in turn is surrounded by a thick-walled fibrous cyst. This cyst is generally oval but varies considerably in size; the largest seen was 0.25 mm. long by 0.175 mm. wide. In this, folded on its ventral face (Fig. 7), lies the metacercaria, and in it the digestive system, ventral sucker, and genital rudiments can be observed. In addition, there is a mass of dark pigment in the excretory vesicle. In the young cyst, remnants of the penetration gland cells and ducts are still visible; these however gradually disappear. When freed from this cyst artificially, a young fluke (Plate III, Figs. 2, 3), about a half-millimetre long, is observed, in which the complete digestive tract, both testes, ovary and a large excretory vesicle filled with this black pigment can be seen.



Apophallus venustus

FIG. 7. *Metacercaria, still enclosed in cyst, but with fibrous outer cyst removed by digestion.*

Fish Hosts

No attempt was made to examine these fish for the presence of metacercariae (other than experimentally infected catfish and the catfish described later) and reliance was placed on experimental feeding of cats. These cats were all raised locally and had not previously been fed on fish. They were placed in cages and each one fed on a single species of raw fish, collected from the Ottawa River.

During 1935 and 1936 the following fish (4) were fed to individual cats:

Scientific name	Popular name	1935 experiments	1936 experiments
Lepisosteidae			
Lepisosteus osseus	Garpike	Positive	—
Catostomidae			
Catostomus commersonii	Sucker, common	Positive	Negative
Moxostoma aureolum	Sucker, red-horse	—	Negative
Cyprinidae			
Luxilus cornutus	Dace	Positive	—
Cyprinus carpio	Carp	Positive	—
Ameiuridae			
Ameiurus nebulosus	Bullhead or catfish	Positive	Positive
Esocidae			
Esox lucius	Northern pike	Positive	Positive
Percidae			
Stizostedion vitreum	Wall-eyed pike (or Doré)	Negative	Positive
Perca flavescens	Perch	Positive	Negative
Centrarchidae			
Micropterus dolomieu	Small-mouth black bass	Positive	Positive
Eupomotis gibbosus	Common sunfish	Positive	Positive
Amblophites rupestris	Rock bass	Negative	Negative

Only seven red-horse suckers were available for feeding purposes in 1936, and so no conclusions can be drawn from the negative results. However, 323 perch were fed with no result in 1936 and the infection from these fish in the previous season was a light one. Forty-nine wall-eyed pike in 1936 gave also a very light infection and it seems reasonable to conclude that these are both "poor" hosts. The number of rock bass fed in 1935 was not recorded but it was large, while in 1936, 43 were fed; in no case did an infection result. This result is peculiar as the closely related small-mouth black bass is an excellent host and the sunfish a good one. The best host however was the common bullhead or catfish, and one cat fed on thirty of these fish yielded 5,882 flukes on post-mortem examination.

Ciurea(3), who has published a very detailed account of the metacercaria of the European *Apophallus donicus* as it occurs naturally in fish in Rumania, finds that it occurs in *Perca fluviatilis*, *Lucioperca lucioperca* and *L. volgensis*

as well as in *L. sandra*, *Percarina demidoffi*, *Acerina cernua*, *A. schraetster*, *Aspro streber* (among the Percidae) and in *Scardinius erythrophtalmus*, *Abramis brama* and *Blicca björkna* (among the Cyprinidae). In light infections, the metacercariae are found exclusively in the fins, while in heavy infections they are also found in the skin or under the scales. In the fins, they are generally in the interior rays and rarely in the membranes; the caudal and pectoral fins are mainly infected. In the skin, they are generally in the deep layers and only in massive infections are they superficial.

The distribution of the cysts of *Apophallus venustus* has only been studied in the catfish. A number of these fish was cut up into carefully separated and identified segments. Each portion was then separately subjected to artificial digestion in pepsin-hydrochloric acid-saline solution for 24 hours. This procedure did not digest away the cyst membrane and the cysts in each part of the fish were easily counted. Their distribution is shown in Fig. 8;

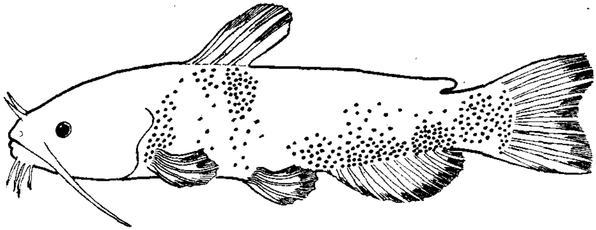


FIG. 8. *Diagram of catfish, showing distribution of metacercariae.*

each dot represents a cyst. The diagram is a composite one, representing the combined results of four separate fish. Most cysts are in the neighborhood of the unpaired, membranous fins (caudal, dorsal and ventral) but some are in the neighborhood of the paired fins as well. No cysts were found in the head region or in the dorsal region near the fleshy dorsal fin. All were in the actual musculature. This distribution agrees with the observations of the actual penetration of young catfish by the cercariae, which were only seen to enter by the membranous fins, never by other parts of the cuticle.

Definitive Hosts

In Canada, this parasite has been found in cats, dogs and raccoons *(Procyon lotor)* as well as in the great blue heron *(Ardea herodias herodias)*. No specimens were found in the following fish-eating birds taken locally under permit from the Department of the Interior:—

8 bitterns *(Botaurus lentigenosus)*.
2 kingfishers *(Megaceryle alcyon)*.
2 gulls *(Larus delawariensis* and *L. argentatus smithsonianus)*.
3 mergansers *(Mergus merganser americanus)*.
1 hooded merganser *(Lophodytes cucullatus)*.

The development in the final host has not been systematically studied. Although artificial digestion experiments suggest that the fluke excysts only after *intestinal* digestion, Ciurea found, on autopsy, that the metacercaria of *A. donicus* after excystment became ovigerous in two and a half days. In one critical experiment carried out here however, eggs were not passed by the cat until the twenty-fourth day after infection, although careful fecal examinations were made daily. However, from a cat fed on experimentally infected catfish, flukes containing eggs were recovered on autopsy, a week after infection.

Several infected cats retained in the laboratory over winter had lost their infection by spring, and it seems probable that the mature life of the trematode is limited to a few months in these animals, and that the infection is maintained in the metacercarial stage in fish. This hypothesis is strengthened by the fact that no infected snails were found locally before September, although large numbers were collected and examined throughout the summer.

Careful examination of the intestines of cats passing ova, showed that the majority of the trematodes were in the ileum, a few were in the jejunum and none in the duodenum. The villi are rather far apart in the latter part of the small intestine of the cat and this may partly account for the relatively short life of the parasite.

Malformations (Plate III, Figs. 4, 5, 6)

In several cases, malformed specimens were encountered, the principal alteration being in the number of the testes. In one case both testes were completely absent, while in several one or other testis was absent. It is interesting to recall that several genera in this family have normally only a single testis, while in others the position of the testes relative to each other is a variable character. In none of the present series was the latter character observed.

Acknowledgments

During the course of this work I have had the assistance not only of my colleagues but of numerous persons outside the Institute, and I wish to acknowledge particularly the help of Mr. J. B. Harkin and Mr. Hoyes Lloyd of the National Parks of Canada, Department of the Interior, who gave us authority to take several migratory birds; Mr. L. A. Richard, Deputy Minister, and Mr. B. W. Taylor, Department of Public Works, Game and Fisheries, Quebec, who gave us authority to net fish; Mr. A. LaRocque, National Museum of Canada, who identified the snails and Professor V. C. Wynne Edwards, McGill University, who identified the birds. I wish also to thank Professor Ciurea of Bucharest, Rumania, who gave me a number of specimens of *Rossicotrema donicum*, Dr. Price of the Bureau of Animal Industry, United States Department of Agriculture, who gave me the benefit of his experience in the systematics of this group, and Mr. J. J. Harpell, of Gardenvale, Que., who gave us permission to collect catfish and minnows from his pond.

PLATE I

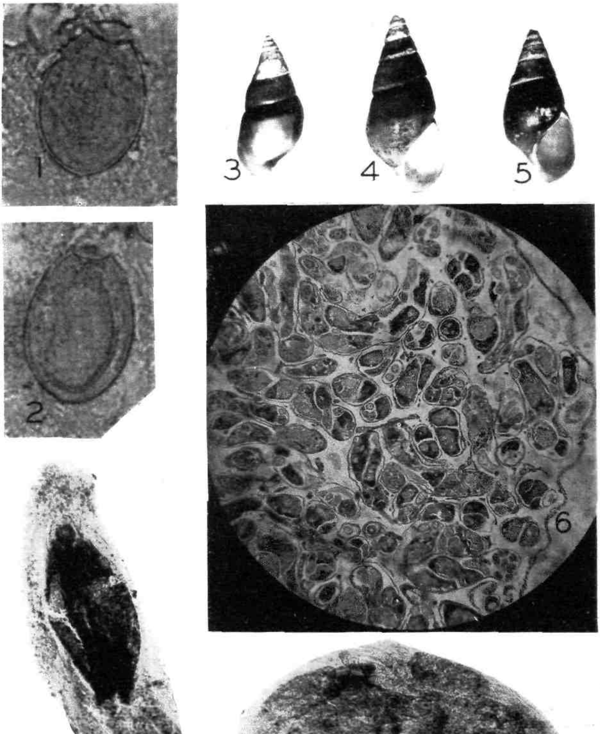
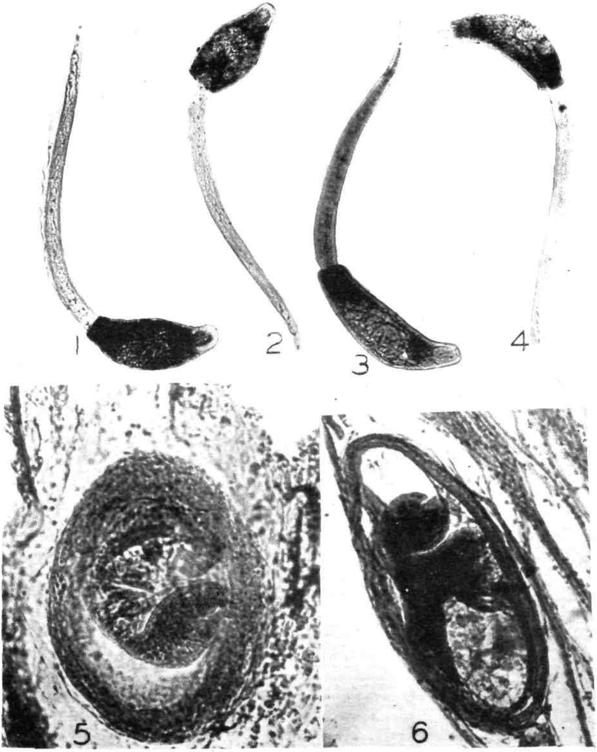


FIG. 1. *Egg of Apophallus venustus as passed in feces of cat (the operculum having been removed by pressure).* FIG. 2. *Egg containing miracidium.* FIGS. 3–5. *Goniobasis livescens.* FIG. 6. *Section of liver of Goniobasis livescens, showing daughter rediae and developing cercariae.* FIG. 7. *Daughter redia with two fully developed cercariae.* FIG. 8. *Daughter redia with eight cercariae.*



FIGS. 1–4. *Cercariae of Apophallus venustus.* FIG. 1. *Cercaria showing flanged tail, in position of rest in water.* FIG. 2. *Cercaria from ventral aspect showing eyespots, glands and genital primordia.* FIG. 3. *Cercaria from lateral aspect.* FIG. 4. *Cercaria from lateral aspect showing ventral sucker.* FIGS. 5–6. *Sections of metacercariae in muscles of catfish (Ameiurus nebulosus).*

PLATE III

FIG. 1. *Metacercaria of Apophallus venustus with fibrous cyst removed.* FIGS. 2–3. *Immature adults from intestine of cat.* FIGS. 4–6. *Malformations in adults.* FIGS. 4 *and* 5 *show absence of a testis and* FIG. 6. *absence of both testes.*

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 15, SEC. D. MARCH, 1937 NUMBER 3

STUDIES ON THE GENUS *RHAGOLETIS* (TRYPETIDAE) WITH SPECIAL REFERENCE TO *RHAGOLETIS POMONELLA* (WALSH)[1]

BY A. D. PICKETT[2]

Abstract

This paper is a report on an investigation of the biological and morphological relations of a number of species of dipterous flies belonging to the genus *Rhagoletis* (Fam. Trypetidae). If the observations made and the conclusions drawn are valid, it would appear that the practice of separating species on the basis of very slight morphological differences, which may be due to host influences, is unsound. The forms which develop in apple, hawthorn and blueberry, were studied extensively from the standpoint of their relations to their hosts and the conclusions reached are that one species *Rhagoletis pomonella* (Walsh) is involved and that the female insects show a decided preference to oviposit in the host in which they developed as larvae.

Introduction

The dipterous insect *Rhagoletis pomonella* (Walsh), variously known as the apple maggot, railroad worm and blueberry maggot, is probably indigenous to eastern North America and has been mentioned in reports of entomologists and horticulturists for many years.

There has long been considerable doubt in the minds of entomologists as to the relationships of the forms found in the different hosts in which the insect breeds. Some authors have regarded the forms that breed in apple, hawthorn and blueberry as belonging to a single species, but of late there has been a tendency to regard the blueberry form as a distinct species and in 1932 (11) it was so described.

Judging from the literature on the subject it appears to be more or less generally accepted that the apple and hawthorn forms will develop in either host and that there are no definite biological or morphological differences between them. On the other hand attempts have been made by a number of investigators to show both biological and morphological differences between the apple and hawthorn forms on the one side and the blueberry form on the other. When the present investigation was undertaken, the writer was of the opinion that some differences actually existed.

In Nova Scotia, where these investigations have been carried on, the relationships of these forms are of considerable economic importance, since in the main fruit-producing area all three host plants are found growing in close proximity and consequently, if there is a free movement from one host plant

[1] *Original manuscript received November 28, 1936.*
Prepared from a Thesis presented at the Spring Convocation of McGill University, 1936, as a partial requirement for the degree of Master of Science.
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to another the difficulty of controlling this pest on fruits of economic importance is increased. It was with the object of clearing up the relationships of these forms that these investigations were undertaken.

As the investigations progressed it occurred to the writer that the study of other closely related forms might be of some assistance in clarifying certain points, especially those dealing with the morphology of these forms. Consequently, specimens of the form found breeding in the snowberry in British Columbia were procured and studied. This form has been described as a distinct species under the name of *Rhagoletis symphoricarpi* Curran. It has not been possible to include this form in the biological investigations, but it would be desirable to do so were sufficient living material available. The forms found breeding in cherry offer very interesting material for study and it is regretted that breeding work with these could not have been included in the present investigation as this would appear to be a problem somewhat similar to that found in the case of *pomonella*.

While it has not been possible to carry out all the breeding work that appears necessary to clear up all the points in doubt, the writer is of the opinion that the results obtained to date indicate fairly clearly what the final outcome will be. It was originally intended to include genetical studies of the forms of *Rhagoletis pomonella*, but time has not permitted this, owing to the great amount of detail involved in the breeding work.

It is hoped that the present report will renew interest in this very interesting group of insects and that it may help to clear up what were, evidently, very erroneous ideas regarding the relationships of a number of forms belonging to this genus. The biological studies are being continued.

General Considerations

In order to determine the relationships of the above mentioned group an investigational program was outlined. This was divided into two main sections: first, the morphology of the forms in question, and also of other related trypetids that were available; and second, a biological study of the forms developing in the apple, the hawthorn and the blueberry. The results of these investigations are given below.

Morphological Studies

Rhagoletis pomonella (Walsh)

The *pomonella* group presents a difficult problem; and after a careful study of a long series of specimens, along with a painstaking survey of the literature, there is still considerable doubt as to the status of the various forms.

Accordiug to Illingworth (20), Porter (29) and Benjamin (4) the species *Rhagoletis pomonella* was described by Walsh (34) in 1867 under the genus *Trypeta;* the writer has not examined the original manuscript. This description refers to the flies developed on the apple and on the hawthorn; and there is no question as to its status. However, when Snow (31) described *zephyria* in 1894, the status of the group became somewhat complicated because the differences between the two forms were comparatively slight. Doane (12)

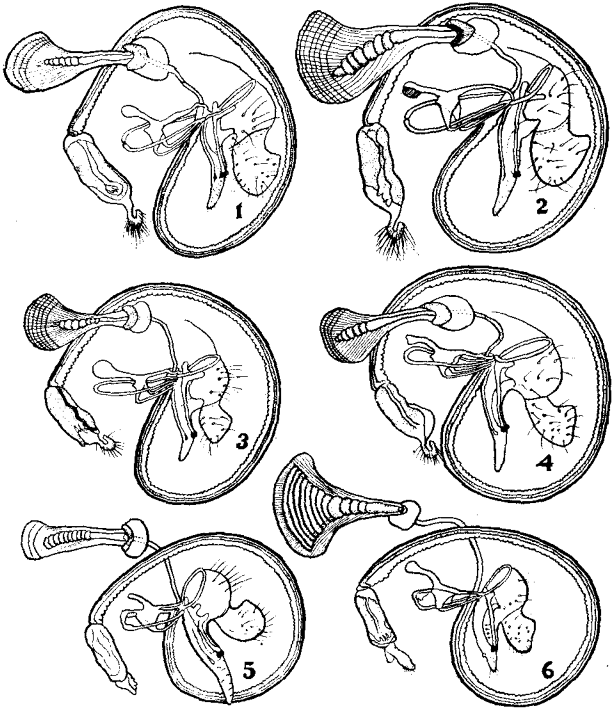
and Aldrich (1) questioned the validity of Snow's species. Curran (9, 10), however, after an examination of one female specimen accepted *zephyria* as a definite species on the basis of color variations between it and *pomonella* (Walsh). A related form, found in the snowberry *Symphoricarpus racemosus* Michx., was described by Curran (9, 10) under the name *symphoricarpi*, on the basis of the characters of the male genitalia. Cresson (8) regards the latter as a synonym of *pomonella*. Benjamin (4) has examined one of the cotypes of *zephyria* Snow, and cannot distinguish between it and those reared from the snowberry; consequently he has designated *symphoricarpi* Curran as a synonym of *zephyria* Snow.

In 1932, Curran (11) examined specimens collected from blueberry, that were submitted to him by the writer for identification, and he described them as a new species *R. mendax*, on the basis of the characteristics of the male genitalia. Woods (35) studied the blueberry maggot in Maine, and after comparing all stages with Illingworth's (20) figures of *pomonella*, could observe no difference except in size and habits. In 1933 a series of specimens collected from apple, hawthorn, and blueberry were submitted to Dr. Alrich of the United States National Museum who, although he was unable to decide definitely (3), was of the opinion that there was not enough difference between the blueberry form and the others to give the former specific rank.

During the last four years the writer has spent a considerable amount of time studying the male genitalia of a number of species of *Rhagoletis*. A long series of the forms collected or reared from apple, hawthorn, blueberry and snowberry, have been carefully examined and a number of figures have been prepared.

There does not appear to be any constant difference in the male genitalia of the forms that occur on apple, hawthorn and blueberry; as a reference to Figs. 1, 2 and 3 will show. Curran's figures (11) are only partially complete; and the specimens were evidently not examined in the same plane. Further, his statements that the so-called sustentacular apodeme (ejaculatory apodeme) furnishes a "ready means of determining the species" and that "this organ exhibits striking differences in shape in the various forms studied" have not been substantiated by the writer's examination of a series in each group. In the specimens examined there is a great variation in the form of the ejaculatory apodeme in each group, and this character cannot be used for the purpose of identification. The writer is of the opinion, although this point was not definitely proved, that this organ develops and becomes larger in accordance with the period elapsing after the fly leaves the puparium. Specimens of apple and blueberry flies that had just emerged showed a weak development of this organ. A careful examination of the ejaculatory apodeme of each form shows that neither the crescentic rings, the width and shape of the apical portion, nor any other feature of this organ can be used to differentiate among these forms. A brief reference to Figs. 1, 2 and 3 will show some differences in the ejaculatory apodemes. These are drawings of specimens chosen at random for this purpose and none of the slight variations are constant. It will be noted that these figures show a greater difference in the size of this

organ between the flies from hawthorn and those from apple than between the apple and blueberry forms; but the difference is not constant. The observations of the writer indicate that the tip of the penis is the most constant feature found in the male genitalia of all the species of the genus studied. As Curran (11) has pointed out, the proper orientation of these structures greatly affects the conclusions that may be drawn. If mounted in liquid glycerine these organs can be readily handled. The figures show the type of apical termination and, so far as can be ascertained from a study of a series of each form, the character is constant.



FIGS. 1 TO 6. *All figures of male genitalia drawn to the same scale.* FIG. 1. *Rhagoletis pomonella (Walsh) (apple form).* FIG. 2. *Rhagoletis pomonella (Walsh) (hawthorn form).* FIG. 3. ***Rhagoletis pomonella (Walsh) (blueberry form).*** FIG. 4. ***Rhagoletis pomonella (Walsh)*** *(snowberry form).* FIG. 5. ***Rhagoletis tabellaria (Fitch).*** FIG. 6. ***Rhagoletis ribicola*** *Doane.*

are not to be relied on. On the basis of an examination of ten specimens reared from each of the fruits, hawthorn, apple and blueberry, the hyaline indentation between crossbands two and three reach the fourth vein in some of each of these forms. In those bred on hawthorn, one specimen out of ten exhibited this condition; in the apple flies four specimens out of ten; and in the blueberry flies seven specimens out of ten showed this character.

The situation regarding the form from the snowberry is slightly different. The writer has not had an opportunity to examine the form reared from the sparkleberry *Batodendron arboreum* Marsh (Nutt) which Benjamin (4) considers to be conspecific with the snowberry form and also with Snow's *zephyria*.

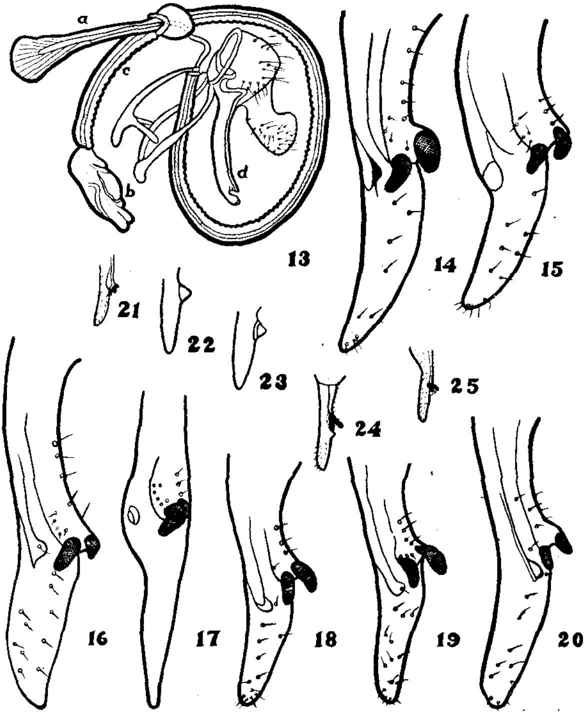
Curran (11), in reaffirming his identification of *symphoricarpi* as a species, states that in his examination of the male genitalia he probably overlooked the most striking differences. Benjamin (5) maintains that the differences in the male genitalia as referred to by Curran (10) are sufficiently constant to separate this form from *pomonella* (Walsh).

The writer has examined the genitalia of a long series of each form and although certain characters mentioned by Curran (10) are fairly constant, even though somewhat variable, he cannot agree that the differences mentioned in Curran's (11) key are constant. Curran's (10) figures, however, are essentially correct in so far as they show the general shape of the claspers; but the statements regarding the lobe of the clasper and the length and shape of the hooks are not borne out by the present studies (Figs. 16–25). In most specimens, however, it is possible with careful orientation to see the more curved condition of the clasper in the apple form; the more ventral position of the apical tip or point of the clasper as compared with the form on the snowberry; and the wider and more compressed condition of the clasper beyond the spurs as seen in the latter. It is necessary to study these forms very carefully to note these apparent differences, as a slight variation in position during the examination entirely changes the appearance of these parts.

If one studied only the forms found on apple and snowberry it would be fairly easy to come to the conclusion that these differences were of sufficient significance and consistency to separate the forms as different species; but when the forms reared on hawthorn and blueberry are considered, some doubt develops. Some of the male hawthorn flies show an even greater divergence from those of the snowberry than do the apple flies. That is, the differences referred to above appear to be somewhat more intense (Figs. 14–16). This does not hold true for all individuals. When forms from the blueberry are studied, certain individuals, probably the minority, show these characters as being nearly midway between the apple and snowberry forms. These characters may be noted by comparing Figs. 14–25. With this additional information, the writer is inclined to believe that the snowberry form should not be regarded as a separate species.

One other feature which exhibits the very close relationship among all these forms is the nature of the tip of the penis, in which there is absolutely

no variation so far as can be found when complete specimens are examined. Among all the well defined species studied by the writer in this genus, with one possible exception, which will be referred to later, there were distinct differences in this part of this organ.



FIGS. 13 to 25. FIG. 13. *Male genitalia of Epochra canadensis Loew. a. Ejaculatory apodeme. b. penis. c. penis tube. d. claspers.* FIGS. 14–25. *Claspers of Rhagoletis pomonella (Walsh).* FIG. 14. *Right clasper of hawthorn form (latero-medial aspect).* FIG. 15. *Right clasper of apple form (latero-medial aspect).* FIG. 16. *Right clasper of snowberry form (latero-medial aspect).* FIG. 17. *Postero-lateral aspect of clasper shown in Fig. 16.* FIGS. 18–20. *Right claspers of blueberry form showing variations in apex.* FIG. 21. *Clasper of apple form showing three hooks.* FIG. 22. *Clasper of apple form showing shape of lobe opposite hooks.* FIG. 23. *Clasper of snowberry form showing shape of lobe opposite hooks.* FIG. 24. *Posterior aspect of clasper of apple form showing hooks and lobe.* FIG. 25. *Posterior aspect of clasper of snowberry form showing hooks and lobe.*

A study of the larval characters which are mentioned by Greene (17) in his study of trypetid larvae was completed. This failed to indicate any significant differences between maggots taken from hawthorn, apple, blueberry and snowberry.

The specimens from snowberry were supplied by Mr. W. Downes, Victoria, B.C.

Rhagoletis cingulata (Loew)

In the past this has been considered a very stable species with clearly defined characters, but in 1932 Curran (11) named two new species from closely related forms. The writer obtained specimens of both of these and dissected the male genitalia for comparison with the form reared from cultivated cherries. After comparing a series of the form which Curran (11) has designated as *indifferens* with a series of *cingulata* there is no doubt in the mind of the writer that the two are identical in so far as the male genitalia are concerned, and that *indifferens* should be considered as a synonym of *cingulata*. The differences mentioned by Curran (11) do not hold; and here again it may be pointed out that the size and shape of the ejaculatory apodeme is so variable in all these forms that it cannot be used as a taxonomic character. Reference to Figs. 7 and 8 will show that in the two forms figured, the form designated as *indifferens* by Curran has a somewhat wider ejaculatory apodeme than has the form from the cultivated cherry. This feature is not constant, however, and varies greatly in the specimens studied. The other differences mentioned, but not specified by Curran (11), were not observed by the writer. It will be noted also by the figures that the tip of the penis, which appears to be a fairly good taxonomic character, is similar in both forms. The findings as cited above agree with the observations of Benjamin (4) on forms reared in Florida.

The specimens of *cingulata* studied were supplied by Prof. L. Caesar, Guelph, Ontario, and Dr. F. L. Gambrell, Geneva, N.Y. The western form from the wild cherry was supplied by Mr. S. C. Jones, Corvallis, Oregon.

Rhagoletis berberis Curran

This is a new species established by Curran (11) on the basis of the nature of the wing banding. The wing pattern figured by Curran (11) appears constant in the specimens examined by the writer. These were from the type locality. Further, an examination of the male genitalia indicates that the species is distinct from *cingulata*. Figs. 7–9 show a number of differences, but the writer considers the nature of the tip of the penis to be of particular significance.

Curran (11) has mentioned the resemblances of this species to *R. completa* Cresson, and there does appear to be a fairly close relationship in both wing banding and male genitalia. The writer, however, is of the opinion that, as the differences found in the latter, as shown in the figures, are fairly constant and as the general body color is much darker in *berberis*, they should be regarded as distinct species.

Curran's types were reared from the fruits of *Berberis nervosa* at Hood River, Oregon, by Mr. S. C. Jones. The writer's specimens were supplied by Mr. Jones, but are labelled as being from grape.

Rhagoletis fausta (Osten-Sacken)

There appears to have been very little confusion about the status of this species since Aldrich (2) established the synonomy of *R. intrudens* Aldrich. A good description of the species is given by Cresson (8) but the male genitalia have not been figured previously so far as can be ascertained. Fig. 10 shows that the male genitalia of this species are quite different from those of the other species studied, which indicates the value of these characters in a taxonomic study of the genus.

Specimens were obtained from Dr. F. L. Gambrell, Geneva, N.Y.

Rhagoletis sauvis (Loew) and *R. completa* Cresson

Rhagoletis sauvis was described by Loew (22) in 1862; and in 1929 Cresson (8) established *completa* as a subspecies. Boyce (5) considers the differences great enough to establish the latter as a distinct species. He figures two characters found in the male genitalia, the validity of which the writer must question. It is true that there are slight differences in the claspers, but these are not as pronounced as shown by Boyce (5) and it would appear that a different perspective was used in each case. Boyce's figure of *completa* compares favorably with Fig. 12, but to get the claspers of *sauvis* to appear like those in Boyce's figure, it is necessary to orientate the part so that a postero-lateral view is obtained. Fig. 11 shows the condition observed by the writer. Further, these studies showed no constant difference in the size or shape of the ejaculatory apodeme, and as pointed out previously in connection with other species, this character cannot be relied upon in taxonomic determinations. A comparison of Figs. 11 and 12 shows these organs as they are found in the two specimens chosen at random for drawing. The distinguishing characters, as illustrated by Boyce (5) may occur in some specimens in either form, but cannot be considered as constant.

A reference to the figures will show the very close relation of the two forms. The differences between these are certainly not marked, the character of the wing pattern being probably the most pronounced. It was not possible to find any distinct differences in the tip of the penis. There may be sufficient justification for establishing *completa* as a subspecies, but in the opinion of the writer there is not sufficient evidence of differences to consider that two distinct species are involved. However, in order to establish definitely the status of those forms, genetical and cross-breeding investigations should be nndertaken.

The specimens of *sauvis* were obtained from Dr. F. L. Gambrell, Geneva, New York, and those of *completa* from Dr. A. N. Boyce, Riverside, California.

Rhagoletis tabellaria (Fitch) and *R. ribicola* Doane

Rhagoletis tabellaria was described by Fitch (13) in 1856 under the genus *Tephritis*. In 1898, Doane (12) described a closely related form found on currant and gooseberry in Washington as *R. ribicola*. Aldrich (1) recognized this as a valid species and figured the wing. In 1915, Marcovitch (23) described a new species which he called *juniperinus* from flies reared on *Juniperus virginiana* in New York state. This was accepted by Phillips (27) as a valid species. The writer has not had an opportunity of studying the latter form, but the wing pattern as figured by Marcovitch (23) and Phillips (27) appears to resemble the form *ribicola* rather than *tabellaria*. Cresson (8) regards *ribicola* and *juniperinus* as synonyms of *tabellaria*. The writer has dissected and studied the male genitalia of one specimen of *ribicola* from Washington and considers it to be quite distinct from the specimen of *tabellaria* studied. Figs. 5 and 6 indicate very distinct differences between these species in regard to the shape and length of the claspers and also the nature of the tip of the penis. It will be noted that there are marked differences in the size and shape of the ejaculatory apodeme in the two figures, but it would require a study of a long series of specimens to determine the value of this character, and these are not available. However, there are such wide variations in these organs in all species where a series was available for study, that this character should be disregarded, at least until proved to be of value. On the basis of this examination the writer considers that *tabellaria* (Fitch) and *ribicola* Doane should both be regarded as distinct species.

Specimens examined were as follows: One specimen of *tabellaria* and one of *ribicola* from Mr. W. J. Brown, Entomological Branch, Ottawa; one specimen of *ribicola* from Mr. J. F. Gates Clarke, Pullman, Washington; one specimen of *tabellaria* from Dr. E. A. Chapin, United States National Museum, Washington, D.C.

Biological Studies

Historical

Walsh (34) described *Rhagoletis pomonella* from specimens reared from apple and hawthorn and therefore, inferentially, he regarded them as being identical. Other authors, so far as can be determined from the literature, have not considered there were differences between the flies that pass their larval stages in the apple and those found in haws.

In addition to the apple and hawthorn, crab apple, pear and plum have been reported as hosts; and Lathrop and Nickels (21) reared this species from the following plants found on blueberry barrens:

Blueberries: *Vaccinium angustifolium* Kalm (m.a.), *V. canadense* Ait. (m.a.), *V. corymbosum* L. (m.a.)

Bunchberry, *Cornus canadensis* L. (m.a.)

Chokeberry, *Aronia melanocarpa* (Michx.) Britton (m)

Huckleberry, *Gaylussacia baccata* (Wang) C. Koch (m.a.)
Mountain cranberry, *Vaccinium vitisidaea* minus Lodd (m)
Dwarf serviceberry, *Amelanchier bartramiana* Roem. (m)
Wintergreen, *Gaultheria procumbens* L. (m)

(m) indicates fruits in which maggots were found, presumably *R. pomonella*. (a) indicates species from which adults were reared.

Whether these flies would change from one host to another has not been studied until recently, although several authors record observations on this point.

Illingsworth (20) published a detailed account of investigational work on *R. pomonella* carried on at Cornell University in 1911 and 1912. He also gives a technical description of all stages of the insect and records data on distribution and host plants. O'Kane (25) gave comprehensive historical and distributional reports, as well as information on the known hosts.

Woods (35) succeeded in successfully transferring very small larvae from huckleberries and blueberries to the fruit of the chokeberry, *Pyrus melanocarpa*, with the result that these individuals completed their development and pupated. He further observed that only in certain localized areas were blueberries infested with this insect, and that in many cases blueberries growing in the near vicinity of severely infested apple orchards showed no trace of the insect.

The same writer (35) failed to induce apple flies to oviposit in blueberries by confining them in cages on blueberries both in the field and laboratory. Attempts were made to transfer half-grown blueberry maggots to apples by inserting them beneath the skin; but these failed to develop. An attempt to induce flies taken on the blueberry to oviposit in apples also proved abortive. He does not state whether he succeeded in getting flies reared on any fruit to oviposit in captivity on that fruit. Wood's (35) conclusion is quoted as follows: "At any rate, the writer is inclined very strongly to believe that biologically at least there are two distinct strains or races of *Rhagoletis pomonella* Walsh, the one breeding in the apple and related fruits and the other in small fruits such as the blueberry and huckleberry. There does not seem to the writer to be any other conclusion which will explain the data given above. Certainly in so far as *Rhagoletis* occurs in Maine, the form on apple and the form on the blueberry are entirely independent." Similar observations and conclusions are recorded by Patch and Woods (26).

Porter (29) says, "Whether the flies which infest the different fruits are all of the same species is open to serious question. The occurrence of the species in fruit of hawthorn in localities in which the apple is free or virtually free from attack, the reverse condition in other localities, the presence of maggots in blueberries in certain restricted areas and in huckleberries in others, and the distinctly different habits of the blueberry flies from the flies in the apple orchard, all point to the possibility that there may be several

distinct species, biological races, or incipient species, which at present cannot be distinguished from one another."

According to Porter (29) the earlier investigators found difficulty in inducing flies to oviposit in cages.

Lathrop and Nickels (21) encountered difficulty in rearing specimens in confinement and, therefore, were unable to carry on cross breeding and other experiments to procure biological data on the relationship of the flies reared on different hosts. They were able to transfer first instar blueberry maggots to the apple and have them develop. From 200 second instar blueberry maggots treated as above, about 20 developed to the pupal stage and one adult was obtained. These were normal in size for the blueberry form. Second instar apple maggots were transferred to blueberries and some of these formed puparia but no adults emerged. The puparia were normal in size for the apple form. They also state that each maggot consumed two or more blueberries during its development. Their concluding paragraph is quoted, as follows: "It seems probable that the blueberry maggot and the apple maggot exhibit an example of incipient species formation, and from an ecological viewpoint the two forms seem distinct and independent."

In 1931, Fluke and Allen (14) reported on an investigation carried on in Wisconsin in which they secured ready mating and oviposition in cages by feeding a mixture of 1-3% yeast in 5% honey water. This greatly facilitated the carrying out of investigations on these insects.

McAlister and Anderson (24) working on the blueberry maggot in Maine used a modification of Fluke and Allen's method. They carried on interbreeding experiments and found mating occurring when virgin females reared on apple were caged with males reared on the blueberry. However, copulation was less frequent when reciprocal crosses were made. No maggots developed from the latter cross, but of the ten apple females used in crosses with blueberry males, seven deposited eggs in blueberries from which 17 maggots hatched, 15 of which matured and pupated.

Methods Used in Breeding Work

During the first two years a fairly satisfactory technique for handling the flies was worked out. This is described as follows:

In the summer, heavily infested fields of blueberries were located and about the time the maggots began to emerge in numbers large quantities of berries were picked for the purpose of obtaiuing flies for the following season's investigations. The berries, in some cases, were spread out over the ground to a depth of one to two inches in a sheltered location and the maggots emerged from these and entered the soil to pupate. In other cases the berries were placed on soil in flats made with wire screen bottoms. These were set in the ground so that conditions would be as nearly normal as possible. When virgin flies were needed for crossing experiments, the flats were removed to the insectary the following June; but when they were used in studying host

relations, wire cages, constructed in a four-sided dome shape and covered with black cloth, were placed over the soil or flats in which the puparia were located. On the top of the cage was placed a small removable wire screen cage. The flies were allowed to enter this as they emerged from the soil, and a shutter made of a thin piece of board or shingle was arranged so that the passageway between the large and small cages could be closed. This allowed for the removal of the small wire cage so that the flies could be taken from it, or so that it could be replaced by another without the loss of the flies.

In the rearing of apple and hawthorn flies, heavily infested fruit was located in the autumn. When the majority of the maggots were nearing maturity the fruit was gathered and taken to the insectary. Here it was placed in boxes with coarse wire bottoms that would keep the fruit in, but would allow the maggots to pass through freely. Underneath these boxes were placed flats filled with ordinary fine garden soil. As soon as all the maggots had emerged from the fruit and entered the soil, the flats were set outdoors in the earth in a sheltered place, and left until the following June.

Just before it was time for the flies to emerge, the pupae were washed out of the soil by floating them in water. They were then put in sand, in wire cages made about 6 in. in diameter and 8 in. high with wooden tops and bottoms. These cages were covered with dark paper, and a $\frac{3}{4}$ in. hole was made in the centre of the top of each cage. A paper cone leading up to this, inside the cage, directed the flies toward the opening. A glass tube connected this cage with a small wire cage about 3 in. by 3 in., constructed exactly as the larger ones, except that the wire screening was not covered. When the flies emerged they were attracted to the light, and consequently crawled up into the smaller cage. These small cages were made with a $\frac{3}{8}$ in. glass tube in the top through which the flies might be fed. The flies were kept in the small cage until there were enough to take to the field, then the cage was removed and replaced by another.

Before any flies had emerged, clusters of fruits of susceptible varieties located in suitable places were selected, and breeding cages placed over them. The breeding cages were made of 20-mesh japanned wire screening. A circular piece of pine about $\frac{3}{4}$ in. thick formed one end. In this two holes were bored, one $\frac{3}{4}$ in. in diameter for putting the flies into the cage, and the other, through which the flies were fed, was $\frac{3}{8}$ in. to hold a glass tube long enough to reach to the inside of the end and project about 1 in. on the outside. To the other end was attached a cotton sleeve. In apples and hawthorns, the end of the limb that bore the fruit to be covered was cut off just beyond the fruit, or in the case of a smaller limb, it was doubled back and the cage pulled up over the fruit and the cotton sleeve attached tightly to the limb above it. Usually another limb was tied to the first to prevent swaying and subsequent damage to the cage. The cages were placed so that they were fairly well protected and not exposed to too much direct sunlight. With the cages on blueberries great care had to be exercised in regard to the latter point. The cages were so close to the ground and the air moved through

them so slowly, owing to the protection afforded by the dense, low-growing vegetation, that a heavy mortality occurred during the bright warm days unless sufficient shade was provided.

When a sufficient number of flies were procured from the emergence cages, or from field collections, they were taken to the field cages in vials or in the small cages described above. In any case, the flies were put into the cage through the large hole in the end. The cages were connected by a piece of glass tubing about 1½ in. long and of the proper diameter to fit into the hole in the end of each. The small cage was then covered with black paper or cloth and the flies moved toward the light, to enter the larger cage. When they were all in, the small cage was removed and the opening in the breeding cage stoppered. The stopper could be easily removed to permit the addition of more flies. Although this method of transferring flies to the field cages was fairly satisfactory, it proved very slow at times, and in many cases the cotton sleeve was loosened and the small cage placed inside and left until feeding time the following day.

During the first two years of the investigations the flies were fed according to Fluke and Allen's (14) formula. In 1933, however, the writer was informed by Mr. James Marshall, Assistant Entomologist, State College of Agriculture, Washington, that Dr. L. B. Ripley (30), working on the Natal fruit fly in South Africa, had used cow's milk along with the Fluke and Allen mixture and had obtained excellent results. Dr. Ripley's letter to Mr. Marshall is quoted in part as follows: "The Fluke-Allen food does not develop eggs in our females and mating very rarely occurs on this diet, but when it is made with 50% milk, egg production is much increased and mating fairly frequent in large cages."

As this information was originally given to the writer verbally, he was under the impression that the percentage of milk was 5% instead of the 50% quoted above. However, the 5% mixture was used with fairly satisfactory results, although the stronger mixture might have been better. The mixture used was 5% honey, 5% milk and 2 to 3% yeast in distilled water. The mixture was made up fresh before each feeding and the feeding was ordinarily done quite early in the day, usually between eight and ten o'clock, as it was observed that the flies appeared to feed more at that time. Small pieces of absorbent cotton soaked in the feeding mixture were inserted into the glass tubes in the ends of the cages. New cotton was used at each feeding, that used the previous day being removed and discarded.

In securing virgin flies for cross-mating experiments, the methods described above were followed until the time the pupae were washed from the soil. In order to isolate each individual, ⅝ in. holes were bored in pieces of 2-in. planed hardwood planks about 4 ft. long. The holes were placed about ½ in. apart each way so that there were several hundred in each plank. Two or three thicknesses of blotting paper, cut to fit, were placed in the bottom of each hole. The pupae were placed one in each cell and a piece of fine wire screening was placed over the top of each and held in place by two tacks.

The planks were covered with damp newspapers to hold the moisture and then stacked up in the insectary. Each morning all flies that had emerged during the previous 24 hr. were removed and the cells containing pupae were moistened by a few drops of distilled water inserted with a medicine dropper. As the flies were removed they were examined and sorted according to sex and host plants and then placed in small wire cages like those described above, and fed. They were kept in the insectary until a sufficient number of each group had been accumulated to start a cage, when they were transferred to the field cages.

In the cross-breeding experiments the females were put in cages on the host on which they developed as larvae. It was thought that if mating did occur the females would be more likely to oviposit in these than in a fruit of some other species. Where males for the crosses were not available from reared material, they were collected from their host plants.

Observations were made each day on the flies in the cages and records kept of any data of interest such as the behavior, copulation, egg-laying, and so forth.

When the flies were all dead, or when there was evidence that the fruit was likely to deteriorate rapidly, the cages were removed and the fruit either deposited over earth in wire cages, or placed in glass vials covered with cheese-cloth. When all the maggots had left the fruit, the earth was sifted and the pupae counted. Later they were placed in earth and put in a protected place out of doors for observation the following year.

The variety of apple used in the 1934 and 1935 investigations was the Bough Sweet. This is a soft, early, sweet variety, which is very susceptible to apple maggot attacks. Gravenstein was also used in the earlier investigations, but this variety is not as susceptible as Bough Sweet.

Breeding Investigations (1931–32–33)

At the time these investigations were initiated there was no definite information regarding the biological relation of the flies developing on the apple, hawthorn and blueberry. In 1931, experiments were undertaken to find out whether the flies which had developed during their larval period in the fruits of one host could be induced to oviposit in another host, and if oviposition did take place, whether larvae would develop in the new hosts.

Since it was generally considered that the apple and hawthorn flies would cross from one host to the other fairly freely, the first experiments were to ascertain whether the blueberry fly would oviposit and develop in apple; this being the more important economic problem in Nova Scotia.

The breeding work undertaken may be divided into two divisions: First, to find out whether flies reared on one host would oviposit on another; and second, to determine whether flies reared on different hosts would interbreed.

TRANSFERRING FROM ONE HOST TO ANOTHER

At Morristown, in 1931, a small Cox Orange apple tree, a variety susceptible to apple maggot attack, was entirely covered with a cheesecloth cage made as tight as possible. It was felt that if a large cage were used the flies would be more contented and more likely to oviposit. A large number of flies, approximately one hundred, consisting of both sexes, were gathered from blueberry bushes in an adjacent pasture and liberated in the cage. No attempt was made to feed these flies, as it was thought they would be able to find sufficient food in such a large cage (approximately 12 × 12 × 10 ft.). Although frequent examinations of the tree were made, none of the flies were ever observed and it was assumed that they either escaped or died. On trees nearby, blueberry flies were confined in cages similar to those described by Fluke and Allen (14) and fed on the yeast-honey-water mixture suggested by these workers. The flies were fed daily and lived on the average from ten days to two weeks. None was ever observed to make any attempt to oviposit and the flies seemed restless in the cages. When the cages were removed no stings were visible on the apples and no maggots were found in the fruits.

In 1932, more extensive trials were carried out. Before any flies emerged, cheesecloth bags were placed over fruits of hawthorns, blueberries, and susceptible varieties of apples. When flies began to appear on these fruits in locations where they were plentiful, they were captured in fairly large numbers and placed in cages on the various hosts mentioned; that is, flies collected from each host plant were confined in cages placed on apple, hawthorn and blueberry. During this year difficulty was encountered in keeping the flies alive for any length of time. Some difficulty was experienced at first in obtaining wire netting fine enough to hold the smaller flies collected from the blueberry, the wire used first being ordinary 14-mesh japanned wire screening. When 20 mesh copper wire was used, the flies did not appear to live a normal period and it was thought that the copper must be toxic to them. Finally, a suitable type of wire was procured, but it was then too late in the season for it to be of value. Difficulty was also experienced, especially in the cages on blueberry, in supplying the proper amount of shade. If the cages were too much exposed to the sun, a very warm day with bright sunshine would produce a high mortality. Similar observations have been reported by McAlister and Anderson (24). However, in spite of the difficulties encountered, the flies in cages on the host in which they developed as larvae appeared quite contented. Oviposition occurred, maggots developed, and puparia were formed. In only one did any crossing occur and that was in the case of the apple fly on hawthorn; and in one of these cages one maggot developed and formed a puparium.

The work carried on in 1933 showed, largely, a repetition of the 1932 results. The flies behaved normally in cages on the host plants in which they developed as larvae but no crossing occurred, and it appeared that if crossing did occur, it was so rare that it might be regarded as accidental. However, the economic importance of the problem was so great that it was decided to carry on the work another year.

Results of Breeding Work (1934–35)

Table I shows the results obtained in the 1934 investigations. It is to be regretted that the data recorded were lacking in some respects, but it might be said also that data of uncertain validity were discarded.

It will be noted that eight of the 12 cages gave negative results. Cage 1 contained flies collected in the adult stage from the leaves of the apple. These flies seemed to behave normally and the apples were heavily marked by egg-laying punctures. It will be noted that 77 pupae were obtained.

Cage 2 contained flies that had developed as larvae in apples and had emerged from puparia in the insectary and were then transferred to the orchard. Why there was no oviposition here cannot be explained. It might be contended that these flies which emerged in the insectary were not as virile as those collected from the trees, but other data do not substantiate this, as the female apple flies used in Cage 3 also emerged in the insectary. In Cage 3 were apple females and blueberry males, and 27 pupae were obtained. This confirms the results of the work conducted in Maine by McAlister and Anderson (24). These flies appeared to behave in a normal way and lived a fairly long time, mating being noted on several occasions. No results were obtained in cages where blueberry or hawthorn flies were placed on the apple.

Blueberry flies collected from the bushes oviposited in Cage 8, and 21 pupae were obtained. Blueberry flies that had emerged in the insectary showed negative results in Cage 9, but the number of flies involved here was small. In Cage 12, apple flies collected from the trees oviposited in blueberries, and six pupae were obtained. The flies in this cage appeared quite contented after being caged for a few days and they were frequently seen feeding on the surface of the berries. The number of pupae collected appeared small compared to the number of berries apparently infested; it is felt that a number of larvae escaped before pupation, as three were found outside the cage. The report of McAlister and Anderson (24) is hereby confirmed, since it was the female apple flies which they reported as ovipositing in blueberries.

There is some doubt in regard to the results of the work on hawthorn, since the fruits were not covered early enough to preclude all possibilities of their being infested. They may therefore be disregarded.

No adults emerged from the cross-bred puparia that were kept over the winter, but this is not regarded as significant, as those reared on their own hosts also failed to emerge. It is thought that environmental conditions during the winter may not have been normal.

Table II shows the results obtained in 1935. It will be seen that information on Cages 28 and 29 is missing; this is due to the unreliability of the data. Some points of interest are as follows: In Cage 10, on blueberry, apple flies collected from the trees gave one pupa. Similar flies on blueberries in Cage 11 produced two pupae. All the crosses on blueberry gave negative results, and flies reared on the hawthorn also failed to develop on blueberry.

TABLE I

Cage No.	Host	Host in which reared		Place of emergence	No. of flies	Noted		Date started	No. of pupae
		Females	Males			Mating	Stings		
1	Apple	Apple	Apple	Apple trees	—	—	Yes	Aug. 9	77
2	Apple	Apple	Apple	Insectary	—	—	No	—	0
3	Apple	Apple	Blueberry	Insectary (females)	—	Yes	Yes	—	27
4	Apple	Apple	Hawthorn	Insectary (females)	Small	—	No	—	0
5	Apple	Blueberry	Blueberry	Collecting cages	—	—	—	—	0
6	Apple	Blueberry	Blueberry	Bushes	—	—	No	—	0
7	Apple	Hawthorn	Hawthorn	Bushes	—	—	No	—	0
8	Blueberry	Blueberry	Blueberry	Bushes	24	—	—	—	21
9	Blueberry	Blueberry	Blueberry	Insectary	Small	—	No	—	0
10	Blueberry	Blueberry	Apple	Insectary	5	—	No	—	0
11	Blueberry	Hawthorn	Hawthorn	Bushes	—	Yes	No	—	0
12	Blueberry	Apple	Apple	Apple trees	—	—	—	Aug. 9	6

TABLE II

Cage No.	Host	Host in which reared		Place of emergence	No. of flies	Noted		Date started	Date removed	No. of pupae
		Females	Males			Mating	Stings			
1	Blueberry	Blueberry	Blueberry	Collecting cages	92	Yes	No	July 15	Aug. 16	0
4 and 4a	Blueberry	Apple	Apple	Insectary	200	No	Yes	July 20	Aug. 20	0
7	Blueberry	Blueberry	Apple	Insectary	62	Yes	No	July 20	Aug. 19	0
8	Blueberry	Blueberry	Blueberry	Collecting cages	49	—	—	July 22	Aug. 19	11
10	Blueberry	Apple	Apple	Apple trees	35	—	Yes	July 24	Aug. 19	1
11	Blueberry	Apple	Apple	Apple trees	44	—	Yes	July 24	Aug. 19	2
13	Blueberry	Hawthorn	Hawthorn	Insectary	114	—	Yes	July 26	Aug. —	0
16	Blueberry	Blueberry	Hawthorn	Insectary	5	—	—	Aug. 1	Aug. 19	0
17	Blueberry	Hawthorn	Hawthorn	Bushes	16	—	—	Aug. 1	Aug. 19	0
31	Blueberry	Hawthorn	Hawthorn	Insectary	80	—	—	Aug. 2	Aug. 24	0
35	Blueberry	Blueberry	Blueberry	Bushes	34	—	—	Aug. 13	Aug. 20	0
2	Apple	Blueberry	Blueberry	Collecting cages	199	Yes	Yes	July 18	Aug. 20	0
3	Apple	Blueberry	Blueberry	Insectary	181	Yes	Yes	July 20	Sept. 4	0
5	Apple	Apple	Blueberry	Insectary	122	Yes	Yes	July 20	Aug. 28	26
6	Apple	Apple	Apple	Insectary	170	Yes	Yes	July 20	Aug. 20	42
9	Apple	Apple	Apple	Apple trees	20	Yes	Yes	July 24	Aug. 20	16
12	Apple	Blueberry	Blueberry	Bushes	52	—	Yes	July 25	Aug. 28	14
15	Apple	Apple	Hawthorn	Insectary	30	Yes	Yes	Aug. 1	Sept. 13	0 (1 dead larva)
33	Apple	Hawthorn	Hawthorn	Insectary	170	—	Yes	Aug. 5	Sept. 2	17
20	Hawthorn	Apple	Apple	Insectary	275	Yes	Yes	July 24	Sept. 13	17
21	Hawthorn	Apple	Apple	Apple trees	77	Yes	Yes	July 24	Sept. 13	9
22	Hawthorn	Blueberry	Blueberry	Collecting cages	114	Yes	Yes	July 25	Sept. 13	0
23	Hawthorn	Hawthorn	Blueberry	Insectary and bushes	70	Yes	Yes	July 26	Sept. 13	0
24	Hawthorn	Apple	Apple	Insectary	160	Yes	Yes	July 29	Sept. 13	59
25	Hawthorn	Hawthorn	Apple	Insectary and apple trees	63	Yes	Yes	Aug. 1	Sept. 13	36
26	Hawthorn	Apple	Apple	Insectary	275	Yes	Yes	Aug. 2	Sept. 13	35
27	Hawthorn	Hawthorn	Hawthorn	Insectary	180	Yes	Yes	Aug. 7	Sept. 13	73
14	Pear	Apple	Apple	Apple trees	55	—	Yes	Aug. 1	Sept. 13	3
18	Pear	Apple	Apple	Insectary	166	—	Yes	Aug. 1	Sept. 13	0
32	Pear	Apple	Apple	Insectary	140	Yes	Yes	Aug. 5	Sept. 13	0
19	Plum	Apple	Apple	Insectary	235	—	(?)	Aug. 1	Sept. 13	0
30	Plum	Apple	Apple	Apple trees	32	—	No	Aug. 1	Sept. 13	0
34	Chokecherry	Apple	Apple	Insectary	140	—	—	Aug. 7	Aug. 30	0

In Cage 5 it will be noted that in the cross between apple females and blueberry males, positive results were obtained and 26 pupae were collected, which further confirms the work of the previous year.

Cage 12 is probably the most interesting of all. Here blueberry males and females collected from blueberry bushes produced 14 pupae in apples. So far as the writer can determine this is the first record of the blueberry form breeding in apple.

In Cage 15 a cross between apple females and hawthorn males on apple gave only one larva, which was found dead in an apple. In Cage 33 hawthorn flies which emerged in the insectary deposited eggs in apples, and 17 larvae developed and pupated.

Apple males and females appeared to behave in a normal way on the hawthorn, and pupae were procured from cages that contained flies that emerged in the insectary, as well as from those collected from apple leaves (Cages 20, 21, 24 and 26). Also, hawthorn females mated with apple males in Cage 25 produced offspring, and 36 pupae were procured.

The blueberry males and females on hawthorn in Cage 22 failed to produce pupae, although evidence of stinging was quite distinguishable and the flies appeared to be quite contented and lived a considerable length of time. An interesting observation made in connection with these flies was that they appeared to have difficulty in penetrating the haws to deposit eggs, and it may be that for this reason no maggots or pupae were found. A similar observation was made in connection with the blueberry flies on apple, although in one cage they did manage to deposit eggs. In Cage 23, containing hawthorn females and blueberry males, negative results were obtained.

Apple males and females were placed in cages on pear, plum and chokecherry, *Prunus virginiana* L. In the last two, negative results were obtained, although the plum is recorded as a host by a number of authors, and the writer has taken larvae from an unknown variety of very early soft plum in Nova Scotia. In one of the cages on pear (No. 14), where flies collected from apple leaves were used, three pupae were obtained. This confirms the observations of Porter (29) and others that larvae of this species are occasionally found in pears.

One interesting observation made in these studies was in connection with the relative activity of the flies on the various hosts. Woods (35), Patch and Woods (26) and Caesar and Ross (6) point out that apple flies are much more sluggish than those found on the blueberry. This is true to a certain extent, but then again the hawthorn flies are more active than the apple flies, and the writer found them more difficult to collect than blueberry flies. It was noted that apple and hawthorn flies could be observed and captured much more easily on a bright, calm day, but on such a day it was difficult to observe blueberry flies, and it is thought that the direct rays of the sun, especially in the middle of the day, are too hot for them and they keep in the shade. They may be observed and captured more easily between eight

and ten o'clock in the morning and from four to six o'clock in the afternoon. The ideal kind of day to make observations on these flies, or to collect them, is a calm, dull, sultry day; and it was observed on more than one occasion that they could be taken with more than usual ease if an electrical storm was approaching.

It was found to be no more difficult to study blueberry flies in the field, when one had learned their habits, than apple flies. When one is looking down into a mass of vegetation it is more difficult to make observations, as the contrasts are much less sharp, than when examining leaves which are somewhat removed from other vegetation, as in the apple.

Discussion of Results and Conclusions

It is difficult to draw definite conclusions from the data obtained, since some of them are conflicting and not entirely clear cut. However, as pointed out by Porter (29), negative results in breeding work are of no significance; since none of these flies were reared successfully in captivity until Fluke and Allen (14) worked out a satisfactory feeding mixture. The writer did not get positive results in crossing from one host to another until milk was added to the diet, and one cannot predict what may happen with further work on diet and improved methods of handling and breeding.

The writer has made many field observations regarding the habits of these flies, that are not enumerated above, but which have been reported on by other authors. When one observes hawthorns absolutely free from maggots year after year growing within a few feet of apples or blueberries that are very heavily infested, and *vice versa*, it is easy to see how the conclusion may be reached that there are some fundamental differences among these flies. However, since it has been possible in some cases, by using improved methods of feeding, to induce them to adopt hosts other than those on which they were reared, may it not be possible to obtain even more striking results if the proper environmental and dietary conditions are found?

Rhagoletis pomonella (Walsh) has generally been considered as an insect with food habits approaching the monophagous condition, and various authors have referred to it as having "strains" or "forms" feeding on and developing in certain specific fruits.

Some writers, particularly Curran (9–11), regard the forms feeding in the blueberry and the snowberry as distinct species. After spending considerable time studying flies and larvae from the different fruits, the writer is of the opinion that only one distinct species is involved, and that there is not sufficient evidence on the basis of biological or morphological interpretations, or both, to prove the existence of more than a single species. It would appear that a reasonable interpretation would be to regard *Rhagoletis pomonella* (Walsh) as an oligophagous insect, in which the "host selection principle", as enunciated by Hopkins (19), has become highly developed. This theory is quoted from Hopkins (19) as follows: "That an insect species which breeds

in two or more hosts will prefer to continue to breed in the host to which it has become adapted." One of the first entomologists to record observations in this connection was Walsh (33) who described the species now under consideration. However, numerous experiments which indicate that this phenomenon is not of rare occurrence in insects have been reported. Craighead (7) carried on investigations for six years with certain cerambycid beetles in the United States. Two of his conclusions that appear to have a bearing on the point under discussion are quoted here:

(i) "In general, the fewer the hosts in nature, the more marked the predilection for a particular host, and *vice versa*;"

(ii) "Continued breeding in a given host intensified the preference for that host."

The work of Glendenning (16) in British Columbia on the satin moth, the observations by Fryer (15) in connection with apple capsids, the work of Thorpe (32) on *Hyponomeuta padella* L. in England, and of Pictet (28) in France, and of others, lend weight to this theory. Until such time as further investigations have disproved this hypothesis, it would appear to the writer a reasonable one to accept.

Acknowledgments

The writer wishes to acknowledge the assistance and advice received from Drs. W. H. Brittain and E. M. DuPorte of Macdonald College, McGill University, under whose direction this thesis has been prepared. Especial mention should be made of the painstaking assistance received from Messrs. S. V. Nichols and M. E. Neary in carrying out the breeding investigations. The Nova Scotia Department of Agriculture has co-operated in making it possible for the writer to spend a considerable amount of time on this project. The assistance of those mentioned as having donated or loaned specimens is also greatly appreciated.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 15, SEC. D. APRIL, 1937 NUMBER 4

CONCEPTS AND MECHANISMS OF RESISTANCE IN HELMINTHIC INFECTIONS[1]

BY THOMAS W. M. CAMERON[2]

Abstract

The relations that exist between the helminths and their hosts cannot be divided into specific groups. Nevertheless, it is desirable that some classification of these relations, together with clear-cut definition of terms, be widely accepted in order that the subject may be discussed intelligibly. A tentative classification is suggested, which distinguishes between (i) compatibility and incompatibility, which refer only to the host environment as it exists before invasion by the parasite; (ii) resistance, of various kinds and degrees, which refers to the reaction of the environment to the *presence* of the helminth; and (iii) tolerance and intolerance, which refer to the reaction of the environment to the *effects* of the helminth.

The science of immunology deals with the reactions of the body to foreign irritants, organic and inorganic; the organic irritants may include both animal and vegetable parasites, as well as their own products and the products of non-parasitic organisms. Until recently, however, the science has largely been studied as a branch of bacteriology or, to a lesser extent, as a branch of protozoology. Within the past few years, the effects of helminth parasites on the host and the response of the host to these parasites have been under investigation in many laboratories, and numerous papers have been written on the subject. The terminology used, however, has been the terminology of the bacteriologist and, in a number of cases at least, this has resulted in some confusion.

The metazoan parasites are very different in their mode of life from the protista and it does not seem to be possible to interpret their effects without reference to their own actions. The host, on the other hand, is the same, and all the evidence points to an essentially identical mechanism of defense in the host. There is a definite need for a terminology which, describing accurately the relation between host and helminth parasite, will avoid confusion with the already established concepts of the bacteriologist and the protozoologist, and yet will enable the pathologist to reconcile all three points of view.

[1] *Original manuscript received November 7, 1936.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Quebec, with financial assistance from the National Research Council of Canada. This material was presented at the second International Microbiological Congress, London, 1936.

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The word "helminth," although it has a certain practical utility, is undoubtedly a bad one to use in scientific terminology, and although every definition lays emphasis on the fact that it is a faunistic term, this has seldom been properly appreciated in discussions on immunity. It is necessary to remember that the helminths consist of several completely unrelated phyla, or parts of phyla, which have become adapted through the process of evolution to live in a parasitic environment. To do this, they have adopted many different devices—and their different modes of life are just as varied as those which exist among the vertebrates which they mainly parasitize. It is accordingly very dangerous to draw conclusions of a general nature from observations on a single species. This is especially so when it is realized that we know practically nothing about the physiological processes of the various species. Yet it would seem to be obvious that a helminth that has a purely blood diet, and one that feeds on tissue, bile, mucus or other substances, require different sets of environmental conditions.

The helminth parasite is quite different from the protozoan or bacterial parasite. It enters the host terrain as a young form, grows to adult life, passing as it does so through several stages, and often undertaking an extensive migration through the host tissues; finally it settles down to adult life, mates, and produces eggs or larvae, all of which must ultimately leave the host before they can continue their development. Outside of the host they undergo a certain number of larval changes before they are infective to a new host.

No known helminth can complete its life cycle in the same individual; sooner or later the eggs or larvae must leave the host, and if they are to survive, sooner or later they must gain access to the same or another host. (Even in such apparent exceptions as *Hymenolepis nana*, the eggs normally leave the intestine in the feces to return by the mouth and pass their larval stages in the villi of the small intestine.) This is the fundamental difference between helminths and other parasites.

Helminth parasites must be considered as a collection of individual units each entering the host separately. Bacterial or protozoal parasites are quite different. One enters the body and there it multiplies—sexually or asexually—until an infinitely large number results. They are at best simple creatures, without the complicated morphology, and consequently complicated growth to maturity, found in the metazoan parasites.

The parasitic life of a helminth is dependent on its finding a suitable environment in which it can develop to maturity and in which it can reproduce successfully. This postulates a number of favorable factors.

Factors in Successful Reproduction of Helminths

(a) Successful Admission to the Host

The host must traverse the ground where the free stages of the parasite are found; it must eat suitable foodstuffs in or on which the larval stages occur; it must be exposed to the intermediate host, and so on. Even slight

differences in habits, *e.g.*, such as exist between a sheep and an ox in eating grass, may make all the difference in the parasite gaining admission. The anatomy of the host, including the thickness of the skin, are factors to be considered under this heading.

(b) Suitable Environmental Conditions in the Host

Once inside the host, the parasite must find a suitable habitat—type of mucosa in the intestine, length of intestine, presence of suitable food, and so on. There is a belief, which is not so strongly held now as it once was, that the temperature of the host is of paramount importance, especially in warm-blooded animals. The temperature in edentates however is by no means constant, and yet no wild animal that I have examined has a larger burden of parasitic worms—including forms found in species with more or less constant temperatures—than has the lesser anteater of South America. The influence of hibernation is not well understood nor is the influence of fever. However, clinical experience has shown repeatedly that a high fever will often cause a large proportion of helminths to leave the body, just as an enteritis will cause many intestinal forms to pass out.

(c) Possession of a Suitable Protective Mechanism Against the Normal Metabolic Processes of the Host

When the parasite lies in the alimentary system, it must possess some means of preventing itself being digested or being passed out by peristalsis, etc.

(d) Absence of a Host Reaction that would Interfere with the Normal Metabolism of the Parasite

This factor applies most obviously to parasites that leave the lumen of the alimentary tract at some period of their life, but it may apply to all helminths. If there is any host reaction, the parasites must be able to resist its effects.

These groups of factors are essential for the proper development of the parasite to full and functional maturity. If it finds the environment suitable for its development, we may define the condition as "compatibility"; if it is unsuitable, it is "incompatibility". If the parasite dies at once or is passed out unchanged, the condition is "complete incompatibility". If it develops normally with no unfavorable results to either itself or the host, the condition is "complete compatibility". However, intermediate stages exist and the parasite may grow to some intermediate extent. This intermediate extent may stop short of full maturity; the parasite may never be able to reproduce. On the other hand, it may become mature but not fully so; the adults may be small, the egg output reduced and so on. To enable these to be distinguished from each other, the first is called "partial incompatibility", the second "partial compatibility". None of these conditions has any connection with a host reaction; they merely define the type of environment that the helminth finds in the host.

In complete incompatibility and complete compatibility, no host reaction can occur, but in both partial incompatibility and partial compatibility, a reaction may or may not be present, and this reaction may be directed towards the *effects* of the parasite or towards the *presence* of the parasite.

The reaction of the host to the presence of the parasite is termed "resistance". When the resistance is sufficiently high to prevent the parasite from reproducing (*i.e.*, producing eggs or larvae) it is termed "absolute resistance". This may act at any part of the parasite's life, prior to the actual production of eggs or larvae and it is possible to conceive of it being so pronounced as to destroy the parasite immediately on entry. This theoretical conception, which may be referred to as "virtual resistance", has the same result as complete incompatibility, but differs from it in that it assumes a reaction on the part of the host; complete incompatibility on the other hand, assumes an unsuitability of environment, not necessarily inimicable to the parasite —it may be the absence of something. When the parasite is able to reproduce, in spite of the host reaction, the resistance is termed "partial resistance"; the life of the parasite must in consequence be, to some extent at least, abnormal.

The reaction towards the effect of the parasite is essentially a repair process, whereas resistance involves an active alteration in the parasite's environment. In our present state of knowledge, it seems desirable to keep these two concepts separate. Where the damage done is of a purely mechanical nature for example, it is possible that the reaction is directed entirely towards the repair of this damage and does not affect the worm itself. If the reaction towards the effects is sufficient to counteract and repair them, it is referred to as "tolerance"; if not, then it is called "intolerance". These terms are comparative and depend on a great number of varying factors and they must grade into each other to some extent; intolerance postulates clinical disease—tolerance, sub-clinical disease with no obvious symptoms.

Compatibility, incompatibility and resistance may all be natural or acquired, but an intermediate form of acquired resistance may exist called "premunition." In this form some parasites may develop—either successfully or unsuccessfully—but so long as they remain alive, resistance is offered to further infection; as soon as they die or pass out of the host, the resistance will disappear.

These terms have been defined as though they were hard and fast divisions; it is improbable that they are. Thus too many worms in a completely compatible host would induce a state of incompatibility through lack of food. Tolerance and intolerance obviously grade into each other and both absolute and partial resistance can exist side by side in the same host towards the same species of worm. Premunition may precede and grade into acquired resistance. Moreover, it is at least doubtful whether complete compatibility, incompatibility or resistance are more than theoretical concepts. However, any particular state is true absolutely, not comparatively, for any individual worm.

This scheme accordingly proposes the recognition of five concepts in the relation between host and helminth parasite.

Compatibility, as it refers to the environment as it exists before the invasion by the parasite, will be discussed first. Resistance, as it refers to the reaction of the environment to the presence of the helminth, will be discussed next, and tolerance, as it refers to the reaction of the environment to the effects of the helminth, will be discussed last.

Compatibility

"Compatibility" expresses the idea that the host environment is suitable for the successful development of the parasite, "incompatibility", that the environment is unsuitable. Compatibility does not imply any relationship with virulence as does the term susceptibility in bacteriology; this follows from the fundamentally different life history. In a susceptible host, bacteria will multiply indefinitely; in a compatible host, the number of worms will depend on the number of infective stages gaining admission. There is no evidence at all that the term virulence can be used in connection with helminths in the sense in which it is used in bacteriology.

Obviously, compatibility and incompatibility can be complete or partial. "Complete compatibility" expresses the theoretical idea that the host environment is completely suitable for the development of the parasite. It postulates the absence of any reaction or resistance to the worm as well as the presence of all the necessary environmental factors and it is exactly equivalent to a state of commensalism. Obviously, however, no host can be infinitely compatible as there must be a mechanical limit to the number of parasites harbored, and so this state must grade into incompatibility. Too many parasites may cause a stunting in growth or a reduction in egg output, merely by their own number and without causing any form of reaction in the host.

"Complete incompatibility" expresses the theoretical idea that the host is completely unsuitable for the development of the parasite. It also postulates no host reaction, as the environmental conditions are unsuitable in nature before the advent of the parasite. It is a form of passive protection, but as the word "passive" is used in bacteriology with an entirely different meaning, it seems inadvisable to use it here.

There must exist, however, several intermediate conditions of environment between complete compatibility and complete incompatibility.

The parasite may grow to maturity and lay eggs, but it may be abnormally small in size, or produce abnormally few eggs or live a shorter time than normal. This is defined as "partial compatibility", and expresses the idea that the host environment is more or less suitable. In this group of conditions would be included all cases where there is a reaction on the part of the host to the presence of the mature parasite, as such a reaction must cause some abnormality, no matter how small, in the life of the helminth. Partial compatibility, however, has no necessary connection with any host reaction.

If the parasite grows to some extent, but does not reach maturity, the condition is one of partial incompatibility. The parasite dies or is passed out because conditions are unsuitable, but not so unsuitable as to cause it to die on entry or to be immediately passed out. This state may or may not be associated with any form of host reaction to the presence of the parasite; it has, however, no necessary connection with any form of resistance.

It is quite possible that both complete states are purely theoretical concepts that do not exist in nature and that the partial states are the only ones which occur.

It is probable also that compatibility or incompatibility may be natural or may be acquired by changes in diet, concomitant disease and so on; the so-called "age resistance" may, for example be an acquired incompatibility in mature life and a natural compatibility in youth. The presence or absence of vitamins, minerals and other dietary factors appears in some cases to have much to do with rendering the environment suitable or unsuitable, although as our knowledge advances we may find that these factors are concerned mainly with resistance.

Resistance

If worms fail to become established in a host, it must be because the environment is unsuitable for their establishment, assuming that the larval stages are normal and healthy and can gain admission. This may be the result of incompatibility, which postulates the absence of something essential for the worm's well-being, or resistance to their presence, which postulates the presence of something produced by the host, which is inimical to the parasites. This something may be normally present in the host or it may be produced only on the introduction of the parasite or after some equivalent stimulus; it seems impossible in the present state of knowledge to differentiate sharply between incompatibility and resistance, and it seems difficult to draw a line between natural and acquired resistance. However, such differences must exist.

It is essential, moreover, to select some criterion of resistance against helminths, as "protection" may be shown by the immediate death of the worm or its death at any subsequent period or by its physiological processes being altered so that its life is shortened, its growth stunted, its egg output reduced or its habitat abnormally situated. On the other hand, the parasite may pass out of the body in an active larval stage capable of developing in another animal, or may pass into a quiescent larval stage which also can develop in another animal.

The picture is still further complicated by the fact that the helminths are active animals and may move away from any unfavorable environmental influence, just as can any other animal. It is difficult, therefore, to say that a larva passed in the faeces after entry to the host, has been subjected to any different harmful stimulus from one which was killed *in situ*.

The most perfect form of resistance against any worm would cause its immediate destruction upon entry. Although this might be called "immunity", that term is already used in bacteriology with a different meaning, and until more is known of the parasitic life of helminths and the body response to them, to avoid confusion, the term "virtual resistance" (which is not the same as absolute resistance) is suggested as preferable. This is especially so because complete incompatibility would have an apparently identical result so far as the result is concerned, yet would involve no special body reaction. However, if as the result of a host reaction to its presence, a certain species of worm in a host caunot produce eggs or larvae capable of continuing the cycle, it would be perfect resistance from the biological point of view and this would seem to be our logical concept of absolute resistance.

In practice, while virtual resistance has yet to be proved, it appears that an absolute resistance can and does exist in some cases, although no host is absolutely resistant to all species of helminths. Many of the examples of absolute resistance however, are undoubtedly attributable to external causes, such as climate, opportunity and habits due to race, age, sex, etc.; the animal simply is not exposed to infection. When it is exposed to infection, sooner or later, it acquires a great variety of helminths. Man himself is the best example, because his parasites have been most extensively studied. He has few that are specific to himself alone. Of these, but one genus *(Enterobius)* is shared with other primates and can be considered as a natural species. Practically all the others that occur in human beings are either identical with, or closely related to, forms found in domestic or other animals. It is difficult to escape the conclusion that exposure to infection from habits—especially food habits and close association—is the main factor that decides whether a given parasite occurs in a given host. We have been badly handicapped in our knowledge of this subject by the fact that study has tended to concentrate on the parasites of man and those animals closely associated with them.

As our range of experience extends we are finding that host-parasite specificity is much less common than is generally supposed. Our present information is that it appears highest among the cestodes—but we know even less of their biology than of that of any other worms. It appears lowest among the trematodes. To mention only a few of many possible examples, the common liver fluke *(Fasciola hepatica)* occurs in practically every mammal from man to marsupial; Swales (6, 7) and Krull (4) have recently found that the related *Fascioloides magna* will parasitize a wide variety of hosts; *Brachylaemus* of the opossum will live in dogs, cats and young chickens; the Opisthorchidae and the Heterophyidae have an even wider range of hosts; *Sticorchis* of peccaries will not only live in pigs, but also in edentates and rodents.

Among the nematodes, forms with intermediate hosts tend to show less host specificity than those with a direct life history. Heavy infestations of *Spirocerca* of carnivores, for example, have been found in such animals as donkeys which have been given the opportunity of feeding on dung beetles

(5), while *Dracunculus* occurs in a wide variety of hosts in various parts of the world. Many different species of nematodes with a direct life cycle have been developed experimentally in other animals; thus Daubney in East Africa has recently reported the increasing success with which nematodes of wild ruminants are becoming established in sheep, while the genera *Trichostrongylus* and *Strongyloides*, among others, have a wide variety of natural hosts.

Moreover, many helminths that have difficulty in developing in a "normal" host, can reach maturity after host dietetic modifications, such as avitaminosis, disturbance of mineral balance, starvation, and so on.

While the data are scattered and very incomplete it would seem that physiological similarity is much more important than blood relationship in connection with helminth resistance. Helminths are subject to the same evolutionary laws as are other animals, and sooner or later a mutant arrives which is capable of living in the new environment. The closer the physiological similarity of the new to the old host, the greater will be the opportunity for this.

It follows, accordingly, that a state of partial resistance must exist in many cases, in which the host reaction, while not sufficiently severe to prevent the worm from reaching sexual maturity, yet prevents it from reaching its full size, shortens its life, or only permits a small number of the larvae which gain admission, to develop. A similar result may, of course, follow partial compatibility, and it is not always possible to differentiate those two states, *e.g.*, *Clonorchis* is abnormally small in cases when a large number is present—probably partial resistance—or when in an unusual host such as a rabbit—probably partial compatibility. A similar effect is observed in dogs with a heavy ancylostome infection, when the egg output per worm is considerably reduced.

Natural and Acquired Resistance

Natural resistance is resistance to original infection and is the result of an inherited constitutional peculiarity of the host which interferes with the normal development of the parasite. It is usually a species resistance and cannot usually, in the present state of our knowledge, be separated from incompatibility. It may be absolute or partial.

Natural resistance does not always prevent the entrance of the parasite. Cercarial dermatitis is an example of the failure of the human body to prevent the entry of the larval stages of schistosomes that normally develop in other animals and never develop to maturity in man; so far as our present evidence goes, the human body appears to be naturally absolutely resistant to these worms. In the case of the human "creeping disease" due to dog hookworms, a similar state of affairs exists, except in the case of *A. braziliense* where the natural resistance is partial instead of absolute.

It follows from these examples that, while much natural "resistance" is obviously incompatibility, some is undoubtedly active, especially in helminths, the parasitic life of which is not restricted to the lumen of the alimentary

canal. The fact that an active natural resistance can exist, means that the host response must differ in different species of animals.

"Breed" resistance or incompatibility is closely related to species resistance or incompatibility. Webster has shown that a breed resistance against bacteria exists in mice (as well as a breed susceptibility) and that it can be enhanced by inbreeding. This resistance is to some extent non-specific, as in addition to mouse typhoid, the response against corrosive sublimate was increased. Ackert *et al.* (1) have found some evidence of a breed resistance against Ascaridia in chickens and there is some evidence of a breed susceptibility to *Haemonchus* in sheep.

"Age" resistance is generally taken to mean a "resistance" which increases with the age of the host in the complete absence of infection. There is a considerable amount of evidence to link it with incompatibility, although some of the examples are really acquired resistance. It is generally shown towards parasites of fairly closely related hosts. The young animal has a more generalized type of physiology than the adult and there is a fundamental similarity between the milk-fed young of all mammals; later, when they assume the feeding habits of the adult, the similarity becomes less marked. Moreover, young animals experimentally eat objects that adults refuse and their skin is more easily penetrated by larvae. Accordingly, they should, on first principles, have a larger range of parasites than have the adults of the same species. However, some workers believe that some factor (or factors) is lacking in the young animal, which develops in the adult as the natural consequence of age, in the complete absence of infection (3).

It is impossible at present to differentiate sharply natural resistance from acquired resistance. A resistance evoked against one species may operate to some extent against another. Natural infections—especially those which have not progressed beyond the larval stages—are difficult to eliminate in practice.

Acquired resistance follows and is due to previous infection with helminths; it may be absolute, but is usually partial. Such evidence as is available suggests that the intensity of such a resistance is more or less directly proportional to the amount of tissue damage. Thus, in sheep (8), resistance to *Haemonchus* and *Nematodirus*—blood-sucking nematodes—is more quickly produced and more active than resistance to *Ostertagia, Trichostrongylus* and *Cooperia*—non-blood-sucking nematodes. Resistance to larval cestodes developing in somatic positions is relatively great. The few cases of artificially acquired resistance of which we have any knowledge are in this last group. Some workers have also found that they can induce a partial resistance in dogs against *Echinococcus* adults, by the injection of larval cystic material.

Premunition is related to acquired resistance but exists only so long as an original infection with the same species is present; true acquired resistance is subsequent to the infection and survives it. However, premunition may precede true acquired resistance, or may exist simultaneously with the building up of acquired resistance. It is possible that at least some cases of premunition

are due to the accumulation of products of the parasites which make the environment unfavorable to new parasites; such an action might be quite independent of any host reaction and should be classed as acquired partial incompatibility. Undoubtedly many of the examples of the so-called premunition against helminths are based on insufficient data. Some, such as *Hymenolepis nana*, are probably not premunition but true acquired resistance, the cysticercoid stage in the villi having caused the resistance, rather than the adults which have developed from them and are still living in the intestine. Premunition, if it applies at all to infections with helminth parasites, must apply to an infection with more than one individual. The original individual, which induces the resistance, must be able to survive a reaction which forms that enter the host subsequently are unable to survive.

Mechanism of Resistance

Although the mechanisms of active resistance against helminths have been little studied, there is no reason to believe that they differ in essentials from those brought into play against any other foreign invaders. Obviously they must vary from helminth to helminth, depending upon the life processes of the particular parasite under consideration. It is probably true that connective tissue cells form the main body defense and that the response is mainly local rather than general. There is no evidence that any worm produces a toxin in the bacteriological sense, and such noxious substances that do enter the body seem to belong to the class of "foreign proteins," and are the result of the normal metabolism of the parasite.

The presence of eosinophile leucocytes in the blood and other tissues is very common in helminthic infections. There is an increasing amount of evidence to suggest that the function of the eosin-staining granules in these cells is to provide an antidote to foreign proteins, although the cells themselves have phagocytic properties, engulfing and digesting organisms and cells; this property appears to be secondary, however, to their property of rendering foreign proteins innocuous. They are accordingly common in anaphylactic phenomena. A local eosinophilia can be produced only in animals showing eosinophiles in the blood. When these are absent, the eosinotactic substances provoke a considerable accumulation of neutral polynuclear cells. Once this local eosinophilia is established, however, it may persist after the disappearance of the general eosinophilia, and during its establishment it may prevent the appearance of a blood eosinophilia by withdrawing the eosinophiles from circulation.

The general body responses against these dissolved substances are apparently quite strictly analogous to those produced in bacterial infections and there is no need to postulate any special mechanism. As in the case of bacteria, it appears to be affected by defects in diet (including avitaminosis). Ledingham points out that while there is little evidence that nutritional defects disturb the antibody-forming mechanism of the body, there is ample proof that resistance both to spontaneous and induced infection, is profoundly

affected. The precise parts played by particular vitamin deficiencies in producing this lowered resistance are not yet clear. Generally restricted diets may act similarly to diets lacking particular vitamins. The normal excitability of the reticulo-endothelial tissue is greatly reduced by defects of diet which lead to loss of weight. There is a considerable amount of evidence to show that this applies equally to helminths as it does to other foreign stimuli.

Hypersensitivity as the result of helminth infections is a relatively common phenomenon, and allergy is probably a protective mechanism—although it may act harmfully—and it is possible that at least some of the symptoms produced by helminths are the result of hypersensitivity to a relatively harmless parasitic protein. Its normal function is possibly to stimulate a rapid attack on the invading organism or substance and either immobilize or destroy it.

The outstanding reaction against worms however is a local fibrous proliferation. It is the usual inflammatory response which follows the local introduction of any foreign stimulant into the connective tissue of any normal host and serves to isolate the parasite and ultimately to destroy it and repair the tissue damage. Whether the destruction is active or passive is still unknown.

While this reaction is similar in all species of animals it varies considerably in detail from host to host and there is a very urgent need for more detailed studies in comparative pathology. By this is meant a study of the reaction of a variety of hosts to a single cause—such as has recently been carried out by Swales with *Fascioloides magna*. Although known as the "giant American cattle fluke," it is not a normal parasite of cattle. It lives normally in the liver substance of various members of the deer family, especially in elk (or wapiti), coast deer, mule deer and Virginia deer. The lesion in the liver of these animals allows the free exit of ova and thus the life cycle is completed in the presence of suitable snails. However, large Bovidae, including cattle, ranging over the same territory as the deer, ingest the infective cysts on grass, etc., and become infested, the liver appearing dark and irregular in outline and individual lesions appearing, similar in external appearance to common abscesses. The tissue reaction to the presence of the flukes in these animals results in a high eosinophilia and the formation of a hard fibrous cyst from which all means of exit for the eggs are occluded. Thus the parasite is completely enclosed even before it grows to maturity, and its destructive migrations and means of reproduction are stopped. Cattle, accordingly, are absolutely resistant to the presence of this parasite. Sheep, on the other hand, are unable to resist the fluke's migration in the liver tissue and the parasite is, therefore, extremely pathogenic to them and, at least in some cases, death of the host appears to precede the maturation of the parasite. Infected sheep livers appear as obviously diseased organs; discoloration, irregularity of outline and fatty infiltration predominating in the picture. Sheep, like deer, are partially resistant to the presence of the fluke, although their reactions to its effects are different.

Tolerance

Only the effects of the parasite on the host are concerned with disease, and these effects may be due to a variety of causes, negative as well as positive. Apart from the abstraction of foodstuffs and the destruction of other organisms that may be of value to the host, helminths may cause traumatic and other lesions, or produce soluble substances in their excretions or secretions, which are harmful to the host; there is no evidence that any produce toxins in the bacteriological sense. There must be a host reaction to these effects, and if this reaction is sufficient to counteract the effects of the parasite, a state of tolerance may be said to exist; if it does not, then the host is intolerant to the effects of the parasite. It implies no necessary resistance to the *presence* of the parasite although the mechanism of tolerance may be identical with that of resistance. On the other hand it may be quite different. Thus, resistance to the effects of blood-sucking nematodes (such as *Haemonchus*) may be shown by unusual activity on the part of the blood-producing organs, while resistance to their presence may be produced by the connective tissue system. Tolerance will depend upon many factors, external as well as internal, and will vary with individuals, age, diet, intercurrent disease, and so on, and will range from complete tolerance to complete intolerance through an infinite variety of stages. Tolerance can probably be best considered as a repair process and there seems to be no necessity for postulating any specific mechanism different from that used to repair the damage to the host by any other agent. When this process involves a reaction to a dissolved agent, there may be alterations in the serum of the host detectable by test antigens. These do not necessarily play any part in the resistance of the body to the presence of the parasite, any more than do local repair processes necessarily play any part in this resistance.

Tolerance thus used is a comparative term and seems only capable of definition if we assume that it represents the maximum position, *i.e.*, that the body defense against the effects of the parasite is strained to its utmost and that any further increase in effect would break down tolerance. However, any lessening of the effect would not affect the reaction and so tolerance must include all stages from those of an infinitely small nature to those which create the maximum reaction. Beyond this theoretical limit—which must be susceptible of infinite variation due to other causes—we have intolerance, and with intolerance we have the beginning of disease. The greater the intolerance the greater the disease, until at its maximum the host dies. Tolerance on the other hand means the absence of any clinical disease and it may even mean the complete absence of any host reaction to the effects of the parasite because the parasite may have absolutely no effect at all on the host. It expresses the balance of the host-parasite association.

Owing to the delicate adjustment between a host and the species of parasite which is normally found in it, there is less probability of disease than when either host or parasite is unusual. During the process of evolution, the ecology of such a parasite has become so adapted that it does the minimum

amount of damage to the host and so to the chances of continuance of its own species. This adaptation includes not only physiological adaptations of the worm to the parasitic existence in the body, but adaptations of the external larval stages, so that, under normal circumstances, sufficiently few gain admission as not to endanger the host, but not so few as to endanger its own species survival. It is only when these conditions are upset that clinical disease results.

When the parasite is in an uuusual host a relatively small number of helminths may cause quite extensive reactions.

Accordingly, intolerance, accompanied by clinical disease is due, as a rule, to large numbers of "usual" parasites or to small numbers of "unusual" parasites in a host. Thus the case of *Fascioloides magna* mentioned above; sheep are very intolerant while the deer tribe are very tolerant to the effects of the parasite.

Relation Between Intermediate Host and Parasite

Many helminths pass part of their larval existence in various animals, called intermediate hosts. In these animals, the parasite cannot (or at least usually does not) reach sexual maturity. There are certain exceptions to this however. In certain species of trematodes for example, eggs may be found in the "cercarial" stage in snails, although there is no evidence that they are normally deposited during this stage. In some cestodes, such as *Taenia solium* in man, the larva may develop to an infective stage in man, as well as in the normal intermediate host, the pig; in this case, however, they cannot develop further and remain as larvae, unless of course, they are eaten by another man.

The host-parasite relation between a larval parasite and an intermediate host would seem to be strictly analogous to that existing between an adult parasite and a definitive host, although the criteria adopted must differ. If for "sexual maturity," the expression "infective stage," is substituted, the concepts of Compatibility-Incompatibility, Resistance, and Tolerance-Intolerance would seem to apply to them also.

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THE PARASITES OF PIGEONS IN CANADA[1]

By M. J. Miller[2]

Abstract

All the helminths found in the domestic pigeon are reviewed and notes on their distribution, location in the host, and pathogenicity are given. Three helminths, *Ascaridia columbae*, *Capillaria columbae* and *Echinostoma paraulum*, have been found in Canadian pigeons and described, *Echinostoma paraulum* being recorded for the first time in North America. The lip characteristics of *Ascaridia columbae*, which until now have been little studied, are described in detail.

The internal parasites of pigeons have been known almost as long as the study of systematic helminthology itself. As far as can be gathered from the available literature, the first mention of a parasite from the domestic pigeon was in 1782 when Goeze recorded *Ascaris teres*. Since then a considerable number of helminths has been described, the three major classes being well represented with members of several families in each class.

A complete list of the helminths that parasitize the pigeon is given at the end of this paper. In the case of those parasites found in other domestic birds as well as in pigeons, the distribution as found in all hosts is given. A few species from the pigeon recorded by earlier authors, and not referred to in the later literature, are indicated by a question mark.

Trematoda

Location in Host

As far as is known all the trematodes of the pigeon are found in the small intestine. The exact position is not constant but usually includes the duodenum and the anterior part of the ileum; however, *Brachylaemus commutatus*, being a caecal dweller in other birds, would most likely be found in the posterior part of the small intestine.

Pathogenicity

By far the most harmful of the trematode parasites in pigeons is *Echinostoma paraulum*. Mönnig (20) states, "Krause and Van Heelsbergen in Europe and Picard in Java have observed deaths in pigeons caused by this parasite. The birds show inappetence, thirst, diarrhoea, lassitude and progressive weakness. At post mortem there is a slight atrophy of the breast muscles and catarrhal enteritis with much mucus, becoming haemorrhagic behind the duodenum."

Echinoparyphium recurvatum causes emaciation and anemia in fowls and would probably produce similar symptoms in pigeons. According to Neveu-Lemaire (24), the presence of *Brachylaemus commutatus* in certain cases causes a hemorrhagic typhlitis. *Hypoderaeum conoideum* is responsible for localized enteritis in ducks and would probably produce the same condition in pigeons. *Apatemon gracilis* has not been reported as being harmful.

[1] *Manuscript received February 8, 1937.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.
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Cestoda

Location in Host

All the tapeworms of pigeons occur in the small intestine. According to Meggitt (19), they are found mostly in the duodenum, *Hymenolepis* nearer the stomach, *Cotugnia* and *Raillietina* farther away. He concludes however that under normal conditions the worms wander from place to place.

Pathogenicity

Generally speaking the cestodes are less harmful to their host than are the other classes of helminths. However, when they become very numerous there is a pronounced effect on health. Meggitt (19), who has carried out considerable work on cestodes of pigeons in Rangoon, writes that extreme emaciation and weakness were the usual accompaniments of a heavy infection. In young birds a heavy infection would probably be fatal.

Nematoda

Location in Host

The nematodes have a wider range in their adaptation to different locations in the host than have the trematodes and cestodes, and they are found from the crop to the posterior part of the alimentary tract. The spirurid worms are usually found in the proventriculus, but may be found in the oesophagus and crop as well. *Acuaria spiralis* is also found in the small intestine. *Ascaridia columbae* is found in the duodenum or just posterior to it. *Eulimdana clava* is found in the connective tissue around the trachea and *E. mazzantii* in the connective tissue of the neck. The remaining two species are found in the small intestine.

Pathogenicity

Of the helminth parasites of pigeons, the nematodes cause the most trouble. The commonest species is *Ascaridia columbae*. Unterberger (1868) was the first to draw attention to the pathogenicity of this parasite. According to Neumann and MacQueen (23) it causes torpidity, loss of appetite, periodic diarrhoea and finally marked wasting, particularly of the pectoral muscles. Death usually occurs at this period. At autopsy, the mucous membrane is distended in patches, more or less large and numerous, engorged with blood, softened or ulcerated and covered with thick mucus. Irwin Smith (14) reports it as being responsible for the death of squabs in New South Wales. Le Roux (17) attributes the death of pigeons in Natal to this parasite. It should be noted however that *A. columbae* is a very common parasite of pigeons, and adult birds, when lightly infected, normally show no symptoms of disease, although when as many as 400 to 500 worms are found in a single bird, as has been reported, they would probably cause grave disorders. The greatest damage is done to young squabs, and among them the highest mortality occurs.

Capillaria columbae is also very common and when present in large numbers is distinctly pathogenic. Neumann (22) quotes Pauly and Zurn as stating that it often causes an intense intestinal catarrh which leads to anemia and

debility. According to Neveu-Lemaire (24) autopsy of an affected bird shows the intestinal mucosa to be grayish, tumefied and covered with striae and petechiae.

Cram (8) reports heavy infections of *Acuaria (Dispharynx) spiralis* as causing severe losses among carrier pigeons of the Signal Corps of the United States Army. Symptoms described by Whitney are droopiness and loss of weight with retention of a ravenous appetite, as well as loss of pigmentation of the iris, probably due to anemia. A post mortem showed great enlargement of the proventriculus, the inner surface being a thick necrotic mass containing large numbers of *A. spiralis*. Microscopically there appeared to be almost complete destruction of the glands and a marked infiltration of the underlying tissue.

Ornithostrongylus quadriradiatus is very often fatal to large numbers of birds. According to Cram (9) 134 worms are the minimum required to cause death. This species was described in 1904 by Stevenson who mentioned it as the cause of death of large numbers of pigeons in Washington. Komarov and Beaudette (16) reporting on three flocks, state that in one, 46 out of 50 birds died; in another, 20 out of 100; and in the third flock of 17 birds, all died because of this parasite. Le Roux (17) reports the death of pigeons in Natal from the same worm. According to Stevenson, when worms are present in large numbers, death is caused by a general disorder of the nutritive function of the host due to bacterial infection of the pierced mucosa and the loss of blood.

In *Tetrameres* spp. a great deal of damage is done by the female parasite which migrates into the wall of the proventriculus causing marked irritation and inflammation. According to Timon-David (29) who studied the histopathology of *T. fissispina* in the pigeon, there is a true digesting away of the glands by the enzyme of the parasite. Neumann and MacQueen (23), quoting Zurn, state that this parasite kills young ducks. It would probably act similarly in pigeons.

Helminths of Canadian Pigeons

In Canada, according to Swales (28), two helminths have been recorded from the domestic pigeon, *viz.*, *Ascaridia columbae* (by Wickware, 1922 and Rayner, 1932) and *Capillaria columbae* (by Rayner, 1932). Rayner's records are both from Quebec.

The bulk of the material examined in the present investigation was supplied by Messrs. Henry Gatehouse and Son, Montreal. It consisted only of the posterior part of the alimentary tract, usually including the stomach, and was received in lots of 60–80 at irregular intervals. As a result the material was starting to decompose and had to be examined rather hurriedly. The remaining pigeons were obtained locally and kept in wire cages in the Institute of Parasitology. They consisted of 22 in all, 11 Homers and 11 Tumblers.

In the Gatehouse material, 232 intestinal tracts were examined and, on the whole, surprisingly few parasites were found. Sixteen echinostomes (*Echinostoma paraulum* Dietz, 1909), 12 larval nematodes and three capillarids were found. The larval nematodes were undoubtedly ascaridias and most likely *A. columbae*. In comparing them with the larval stages of *A. galli* as recorded by Ackert (1) they approximated the second- and third-stage larvae. The third-stage larvae were 6.3 mm. long; the average length of this stage in *A. galli* is stated by Ackert to be 6.4 mm. Other characters were similar to those described by Ackert. Three distinct lips are present, showing papillae (Fig. 17). In the male there is a distinct pre-anal sucker as well as certain caudal papillae (Fig. 16), and several cells comprising the primordium of the spicules can be seen. The second-stage larvae are 3.8 mm. long; Ackert figures *A. galli* second-stage larvae as 4.2 mm. long. They have lips just beginning to form, an anal prominence, but no pre-anal sucker.

Of the pigeons kept at the Institute, eight were autopsied; all but one harbored capillarids and all but three, ascaridias. The incidence of the infection is given in Table I. Fecal examinations showed that the 11 Tumblers as well as a third of the Homers were infected with ascaridias. It must be remembered, however, that in some cases a pigeon may harbor a single male and consequently not show eggs in the faeces. In the case of capillarids, fecal examinations are not as satisfactory, as the eggs appear sporadically. However, at least three quarters of the birds were infected with capillarids.

The ascaridias were identified as *A. columbae* and the capillarids as *C. columbae*.

The fact that from the Gatehouse material 12 larval ascaridias were obtained, and no adults, suggests that probably a definite age resistance to *A. columbae* is shown by the pigeon, as is shown to *A. galli* by the fowl (Ackert *et al.* (3)).

The pigeons exhibit some evidence of a strain resistance to *A. columbae*. This is suggested by the higher proportion of infected birds among Tumblers than among the Homers, and the fact that

TABLE I

PIGEON PARASITES FOUND

Date		No. of pigeons	Parasites
Nov.	26	66	16 echinostomes 2 larval nematodes
Dec.	6	8	Negative
Jan.	18	78	1 echinostome 10 larval ascaridias 3 capillarids
Mar.	2	1	3 capillarids 1 ascaridia
Mar.	2	1	1 capillarid 10 ascaridias
Mar.	3	1	1 capillarid 2 ascaridias
Mar.	3	1	Negative
Mar.	26	1	1 ascaridia 7 capillarids
Mar.	28	1	3 capillarids
Apr.	17	1	7 ascaridias 1 capillarid

one of the Tumbler pigeons yielded a larva about 4.0 mm. in length, whereas in the Homers experimental infection did not produce a larva over 48 hours (about 0.36 mm.).

Description of Parasites

Ascaridia Dujardin, 1845

Generic diagnosis (From York and Maplestone). Generally with cuticular lateral flanges: oesophagus club-shaped, but without a posterior bulb. Male: pre-anal sucker slightly prominent with a chitinous rim; caudal alae narrow; papillae relatively large; spicules equal or subequal; gubernaculum absent. Female: vulva near the middle of the body; uteri opposed. Oviparous eggs with a thick shell.

A. columbae Gmelin, 1789

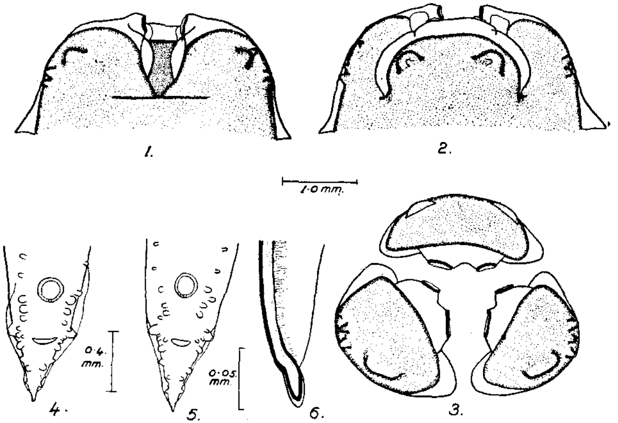
Specific description. There is a great variation in the length of *A. columbae* as given by different authors. The length ranges from 16–25 mm. for the males and 20–35 mm. for the females, as stated by Neumann and MacQueen (23), to 60–70 mm. for the males and 70–90 mm. for the females, as stated by Baylis and Daubney (6). The lengths noted by other authors fall between these two extremes. It should be noted that this parasite occurs in a variety of hosts, and Cram (7) suggests that the variation in length may be associated with this fact. In my specimens the males range from 35 to 42 mm., the females from 43 to 54 mm.

At the anterior extremity are two semi-elliptical membranes, projections of the cuticle. They are about the same length as the pharynx and are marked for part of their width with continuations of the striations of the body cuticle. The pharynx is 2.5 to 2.62 mm. long.

The number of caudal papillae in the male has been given by different authors as 10 to 14 pairs. Johnston (15) figures 13 on one side and 14 on the other. Irwin Smith (14) figures 13 pairs, Yamaguti (31) 13 pairs, and Baylis and Daubney (6) 14 pairs. In the material studied 14 pairs seems to be the normal number, but it is not unusual to find papillae missing or duplicated. The normal arrangement appears to be five pairs of post-anal papillae, of which the third pair is small and inconspicuous and in certain individuals appears to be lacking, a condition which Baylis and Daubney also noted. There is an adanal group of four pairs, one large lateral and three smaller, more ventral pairs. No changes in the number of these papillae were noted, but there are slight variations in different individuals in the relative positions of the three more ventral pairs. Between the anus and the genital sucker are three pairs of rather large papillae. It is not uncommon to find an extra papilla, that is, four on one side and three on the other, and in one specimen there are only two on one side and three on the other. Near the level of the anterior margin of the sucker is a pair more ventrally placed, and anterior to that is found the final pair. The last two pairs mentioned are quite small and inconspicuous. In one of the specimens examined the last pair appeared to be missing; according to Baylis and Daubney, it may be duplicated.

In the male, the anus is 0.44 to 0.48 mm. and the posterior margin of the genital sucker 0.58 to 0.72 mm. from the tip of the tail. The genital sucker varies in outline from circular to sub-circular and sizes noted were 176 × 176μ, 184 × 175μ and 176 × 169μ. The spicules varied from 1.05 to 1.72 mm. in length and were 57μ wide. The proximal part is slightly expanded and the distal extremity tapers abruptly to a blunt point; the posterior part has a cuticular expansion on the inner surface (Fig. 6).

In the female the anus is 0.9 to 1.1 mm. from the tip of the tail.



FIGS. 1–6. *Ascaridia columbae.* FIG. 1. *Head, ventral view.* FIG. 2. *Head, dorsal view.* FIG. 3. *Head, front view.* FIGS. 4 AND 5. *Caudal papillae of male.* FIG. 6. *Tip of spicule.*

Although the lip structures are of considerable taxonomic importance they have been accorded little attention in *A. columbae.* The lips are similar to those of *A. galli*, as described by Ackert (1), that is, three prominent lips, one dorsal and two subventral, the distal margins of each being divided into three lobes, one medium and two lateral. However, where in *A. galli* there is a dentigerous ridge on the inner surface of each medium lobe, in *A. columbae* the inner surface has two saucer-shaped sucker-like structures, placed in such a position that there are three pairs, the members of each pair directly opposing one another (Figs. 2 and 3). They probably function as a hold-fast organ. In reference to the labial papillae, Johnston (15) mentions two small papillae on the dorsal lip. Yamaguti (31) states that, "the three lips are 0.07 to 0.11 mm. long and bear each two papillae, those of the dorsal lip being smaller and inconspicuous." A careful study of the labial papillae showed that there

are two large papillae on the dorsal lip (Fig. 2); on each of the ventral lips there is one large, ventrally placed papilla, and a group of three smaller ones more dorsally placed (Fig. 1); in one specimen the dorsal group was found to consist of four papillae. This suggests that the number of this group may vary, as in the case of the caudal papillae of the male.

Very characteristic of *A. columbae* is the presence of large vesicular-like structures that fill the interior of the body of both sexes. Dujardin and Irwin Smith who appear to be the only authors to mention them, refer to them as "orbicular corpuscles" and consider them to be present only in the female.

Baylis and Daubney (6) mention 26 to 30 pairs of cervical papillae. I have been uuable to recognize them.

The eggs vary in size from 78 to 84μ long by 49μ wide.

Capillaria Zeder, 1800

Generic diagnosis (From York and Maplestone). Body capillary; mouth simple; cuticule with bacillary bands dorsal, ventral or lateral in position; oesophagus long and gradually increasing in size posteriorly. Male: anus terminal or sub-terminal, small membranous caudal alae or bursa-like structure present or absent; spicule long and slender, with or without spines on its surface. Female: vulva near termination of oesophagus. Oviparous eggs lemon shaped, with the usual opercular plugs at the poles.

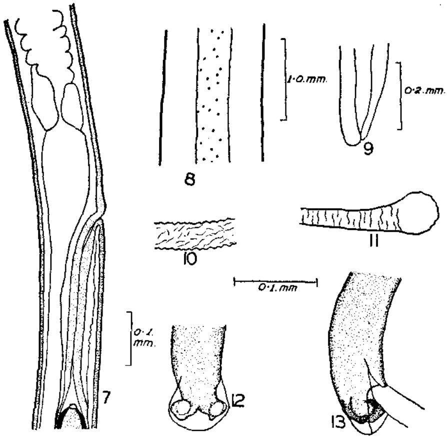
C. columbae Rudolphi, 1819

Specific description. Long thread-like worms with a white translucent appearance; cuticle finely striated transversely; two broad lateral bacillary bands which thin out at both ends, the surface of the bands being irregularly dotted with black spots; cells of the oesophagus rectangular, annulated, with a prominent nucleus in the centre; at the region where the oesophagus and the intestine meet are two smaller non-annulated cells on each side of the digestive tube (Fig. 7).

Male: Only one entire male was recovered. It measures 11.4 mm. in length. According to other authors the length may vary from 8.1 to 11.7 mm. The oesophagus is slightly less than half the entire length or 5.53 mm. The cloacal aperture is nearly terminal and is surrounded by a membranous bursa-like structure. Supporting this membrane are two broad rays, each of which appears to have a secondary outgrowth (Figs. 12 and 13). The spicule in two specimens measures 1.56 mm. and 1.4 mm.; it has a blunt, rounded point and at the proximal extremity expands like a trumpet (Fig. 11). In the entire male specimen the spicule sheath is extruded and measures 2.94 mm. It is irregularly annulated and covered with fine markings (Fig. 10). At the widest part it measures 0.034 mm.

Female: Three females measured 14 mm., 15.1 mm. and 19 mm. This is greater than the lengths recorded by other authors. Graybill (13) mentions 10 to 12.3 mm., Irwin Smith (14), 13 to 16.24 mm. and Morgan (21) 10.1 to 14.5 mm. as the lengths for the females of *C. columbae* they examined. In two females the oesophagus is slightly more than one third of the total length, or 39%. The posterior extremity tapers to a blunt point with the cloacal

aperture slightly sub-terminal (Fig. 9). The vulva is situated near the junction of the oesophagus and the intestine, and has slightly protruding lips (Fig. 7). The vagina passes backwards.



FIGS. 7–13. *Capillaria columbae.* FIG. 7. *Female, in region of vulva; lateral view.* FIG. 8. *Lateral view showing bacillary band.* FIG. 9. *Posterior extremity of female.* FIG. 10. *Anterior end of spicule.* FIG. 11. *Spicule sheath.* FIG. 12. *Posterior extremity of male, ventral view.* FIG. 13. *Posterior extremity of male, lateral view.*

The eggs are ellipsoid, brownish in color but often appear greenish when collected in the feces. They have a thick shell discontinued at either pole where two opercular plugs are present. Egg sizes vary from 47 to 72μ long and 24 to 31μ wide.

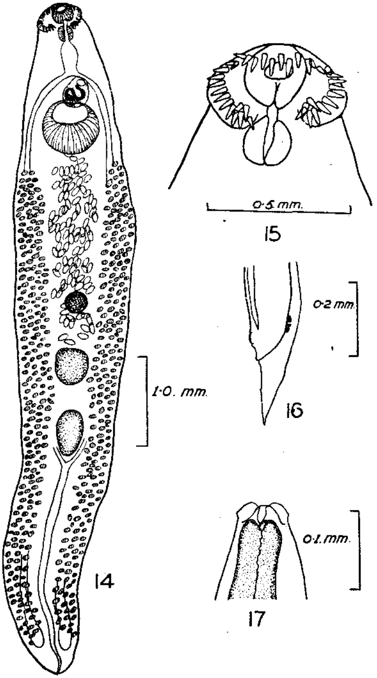
Echinostoma paraulum (Dietz, 1909)

This appears to be the first time *E. paraulum* has been reported in North America.

In 1925 Zunker (35) described this parasite from the pigeon as new under the name *Echinostoma columbae.* Later Mönnig (20) states it to be a synonym of *Echinopharyphium paraulum* Dietz, 1909. Unfortunately I have been unable to see the original description by Dietz, but the characteristics revealed by my specimens, although resembling Zunker's description, would not place them in the genus *Echinoparyphium.* According to Dietz (10) ". . . . im Gegensatz zu allen andern von mir unterschiedenen Echinostomidengattungen sind jedoch die Randstacheln beider Reihen nicht gleich gross, sondern die Stächeln der oralen Reihe kleiner als die der aboralen." While it is true that on the average the aboral spines are longer than the oral spines the difference

is very slight, and as there is an overlapping of sizes, I believe that the trematode should be kept in the genus *Echinostoma*. Therefore, as I consider *Echinostoma columbae* to be a synonym of the echinostome described by Dietz, the name should be changed to *Echinostoma paraulum*.

Specific description. Elongate worm measuring from 4.7 to 8.5 mm. in length. In my specimens the greatest width occurs immediately posterior to the acetabulum and varies from 1.06 to 1.3 mm. In Zunker's figure the greatest width is in the posterior part of the body. The head crown bears two ventral groups of five spines, the arrangement of which is not constant,



FIGS. 14–15. *Echinostoma paraulum*. FIG. 14. *Ventral view*. FIG. 15. *Ventral view of anterior end, showing head crown*.
FIGS. 16–17. *Ascaridia columbae*. FIG. 16. *Posterior extremity of third stage male larva*. FIG. 17. *Anterior extremity of third stage larva*.

and 27 marginal spines (Fig. 15). On the dorsal surface the spines tend to form two rows, and the length of the aboral spines varies from 68 to 80μ while those of the oral are from 66 to 70μ. No spines are found on the body, but, as has been suggested by Mönnig (20) in reference to Zunker's specimens, they may have been lost during post mortem changes. The oral sucker is subcircular and is 0.17 to 0.22 mm. in diameter, the ventral sucker 0.52 to 0.64 mm. and the pharynx 0.17 to 0.22 mm. The oesophagus is 0.36 mm. long and bifurcates halfway between the pharynx and acetabulum. The two digestive caeca reach to near the posterior tip of the body.

The testes are usually oval to circular in outline but may be lobed, tandem, and lie in the third quarter of the body. Zunker (35) states that in the mature specimens he examined, the testes were constricted in the middle. The cirrus pouch is found between the point of bifurcation of the oesophagus and the acetabulum, usually partly overlapping the latter. The ovary is subcircular, located just anterior to the anterior testes. The uterus is a long convoluted tube. The vitellaria are extensive and reach from the posterior border of the acetabulum to near the posterior end of the body. The eggs are oval, and are 96 to 98μ long by 56 to 64μ wide.

The excretory system is Y-shaped with a long narrow base bifurcating immediately caudad of the posterior testis, the two branches running forward to the posterior border of the acetabulum.

In general, the characters as described above agree quite consistently with those stated by Zunker. The differences in shape of body and outline of the testis are not constant characters and therefore could not form the basis for the description of a new species.

Check List of Parasites of Pigeons

Parasite	Distribution
Trematoda	
STRIGEIDAE	
Apatemon gracilis Rudolphi, 1819.	?
HARMOSTOMIDAE	
Brachylaemus commutatus Diesing, 1855.	South Europe, North Africa, England, Indo-China.
B. mazzantii Travassos, 1917.	Brazil.
ECHINOSTOMIDAE	
Hypodaraeum conoideum Bloch, 1782.	Europe.
Echinoparyphium recurvatum Linstow, 1873.	Europe.
Echinostoma paraulum Dietz, 1909.	Europe, Java, Canada.
E. robustum Yamaguti, 1935.	Japan.

Check List of Parasites of Pigeons—*Concluded*

Parasite	Distribution
Cestoda	
BOTHRIOCEPHALIDAE	
Diphyllobothrium crinacei (Rudolphi, 1819), 2nd stage larvae.	?
ANOPLOCEPHALIDAE	
Aporina delafondi Railliet, 1892.	Asia, Africa, Europe, South America, North America.
DILEPIDIDAE	
Choanotaenia infundibulum Bloch, 1779. ?.	World-wide.
HYMENOLEPIDAE	
Hymenolepis (Hymenolepis) serrata Fuhrmann, 1906.	Europe.
H. (Hymenolepis) serrata var. *birmaniea* Meggitt, 1924.	India.
H. (Weinlandia) columbae Zeder, 1800.	Europe, India, North America.
DAVAINIIDAE	
Cotugnia cuneata var. *tenuis* Meggitt, 1924.	Burma, India, Egypt.
C. cuneata var. *nervosa* Meggitt, 1924.	Burma, India, Japan.
Raillietina (R.) micracantha Fuhrmann, 1909.	Africa, Europe, Asia.
R. (R.) nagpurensis Moghe, 1925.	India.
R. (R.) torquata Meggitt, 1924.	Burma.
Raillietina Skrjabinia bonini (columbae) (Megnin, 1899).	England, Europe.
R. (R.) clerci Fuhrmann, 1920.	Asia, Africa.
R. (R.) joyeuxi Lopez Neyra, 1929.	Spain.
R. (R.) tunetensis Joyeux & Houdemer, 1928.	Tunis, India.
R. (R.) taiwanensis Yamaguti, 1935.	Japan.
R. (R.) echinobothridia Megnin, 1881.	Cosmopolitan.
R. (R.) volzi Fuhrmann, 1905.	Asia, Sumatra.
R. Fuhrmanetta crassula Rudolphi, 1819.	Europe, Africa, North America, South America.
Nematoda	
HETERAKIDAE	
Ascaridia columbae Gmelin, 1789.	North America, South America, Europe, Asia, Africa, Australia.
SPIRURIDAE	
Acuaria (Dispharynx) spiralis Molin, 1858.	North America, South America, Europe, Asia, Africa.
Tetrameres (Tetrameres) fissispina (Diesing, 1861) Travassos, 1914.	North America, Europe, Asia, Africa.
T. (T.) confusa Travassos, 1919.	South America.
TRICHINELLIDAE	
Capillaria columbae Rudolphi, 1819.	North America, South America, Asia, Africa, Australia.
FILARIIDAE	
Eulimdana clava Wedl, 1855.	Europe.
E. mazzantii Railliet, 1895.	Europe.
STRONGYLIDAE	
Syngamus trachealis Siebold, 1836 ?	Europe.
TRICHOSTRONGYLIDAE	
Ornithostrongylus quadriradiatus Stevenson, 1904.	North America, Australia, Cuba, Natal.

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The following helminths have been experimentally introduced into the domestic pigeon.

TREMATODES:— *Brachylaemus fuscatus, Echinostoma erraticum, E. exile, E. parvum.*

NEMATODES:—*Oxyspirura parvovum, Tetrameres (Microtetrameres) helix, Trichinella spiralis.*

Acknowledgments

This investigation was carried out under the supervision of the Director of the Institute of Parasitology. It is with pleasure that I express my thanks to the Director and the staff, for their invaluable advice and assistance. I would also like to express my appreciation to Messrs. Henry Gatehouse and Son for supplying pigeon material and to Mr. Louis Meyer for supplying the living pigeons used during the course of the investigation.

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Canadian Journal of Research

Issued by The National Research Council of Canada

VOL. 15, SEC. D. MAY, 1937 NUMBER 5

THE EXPERIMENTAL INFECTION OF PIGEONS AND POULTRY WITH *ASCARIDIA* AND *HETERAKIS*[1]

By M. J. Miller[2]

Abstract

The results of investigations on the intertransmission of pigeon and fowl parasites are presented. They show that *Heterakis gallinae* and *Ascaridia galli* from the fowl are not transmissible to pigeons. Larval *Ascaridia columbae*, however, will develop in young chicks to a 96-hour stage, although they are not present 120 hours after infection.

It is not uncommon for species of parasites known to infect pigeons to be found infecting other domestic birds. It is interesting, however, to note that the cestodes of pigeons are rarely, if ever, found in any of the other domestic birds.

As far as is known very little work has been done on the experimental infection of domestic birds with pigeon parasites or *vice versa*. The only record found was a successful infection of poultry with *Capillaria columbae* from the pigeon (2). Consequently attempts were made to introduce *Ascaridia columbae* into the fowl, and at the same time, *A. galli* and *Heterakis gallinae* into pigeons.

The eggs of the pigeon parasites were collected from the feces of pigeons confined in cages in the Institute of Parasitology, and from gravid female parasites removed after autopsy. *H. gallinae* were obtained from fowl intestines supplied by Messrs. Henry Gatehouse and Son. In the case of *A. galli*, a single gravid female recovered from a fowl egg provided a sufficient supply of eggs.

The pigeons were all examined on arrival for internal parasites, and those used in the experiments were given three successive fecal tests, at intervals of 48 hours, before the date of infection. If, after these tests, the feces showed no trace of helminth eggs, the bird was considered to be free of helminths.

The chicks used in the experiment were secured from the Macdonald College Poultry Department on February 5 as day-old chicks, and were placed in a brooder, all parts of which had been thoroughly sterilized with steam. Later they were moved to wire cages, each cage containing only those birds concerned in one particular experiment. All experimental birds were banded to eliminate confusion in identification.

[1] *Manuscript received February 8, 1937.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council.

[2] *Graduate Student.*

Two methods were employed in the culturing of parasite eggs. In one an antiseptic substance was added to an aqueous egg culture to keep down molds and bacteria, and in the other, a lowered humidity was used for the same purpose. Both methods proved to be satisfactory.

The infective eggs to be fed were drawn off with a pipette and deposited on a filter paper folded in a funnel. In this way the liquid passed through, while the eggs remained on the filter paper. The part of the filter paper bearing the eggs was then cut out, mixed with chick mash, and rolled into a small pellet. With the aid of forceps this pellet could easily be placed in the throat of the bird, which quickly swallowed it. Towards the end of the investigation the filter paper with the eggs on it was fed without mixing with the mash. This was a much quicker method and quite as efficient. *A. columbae* eggs, which were collected in the feces, were passed through fine bolting silk. This separated the eggs from the larger fecal particles. The eggs were then fed in the manner described above.

Experiment 1

Artificial Infection of Pigeons with A. galli

On January 29, a gravid female of *A. galli* was dissected; the uterus was removed and teased out on a plate of glass which was placed in a closed jar at room temperature, with a relative humidity of 97.5%. By February 9, vermiform larvae could be seen moving within the shell. Whether the eggs were infective could not be determined, but in order to make sure, they were left considerably longer. On February 24, a fungus growth was noticed on the eggs, which were then washed off and stored in a small Petri dish with about 1% formol solution. This killed the fungus.

On February 26, two pigeons, which had been previously proved ascaridia-free, were fed about 150 infective ascaridia eggs. Infection was repeated on March 1, 4, 8, 11, 15, 18 and 22. At the same time, in order to determine whether the eggs were infective, two chicks, approximately three weeks of age, were removed from the brooder and placed in a small cage away from the others. In order to take into consideration the possibility of an age immunity in pigeons to ascaridia, two pigeons known to be ascaridia-free were infected with eggs of *A. columbae*, and the infection repeated on the same dates as the infection with *A. galli*. Two ascaridia-free pigeons were used as controls.

On March 24, one of the pigeons infected with *A. columbae* was killed and the intestinal contents thoroughly examined. In the posterior portion of the gut one small larva was found. This was presumably a 48-hour larva, that corresponding with the time since the last infection; it also corresponded to the 48-hour larvae found in other experiments.

The other pigeon infected with *A. columbae* was killed on March 26, having been fed infective eggs 17 hours previously; again only one larva was recovered. These results suggest a strong age immunity in pigeons to *Ascaridia columbae*. This age immunity would probably affect *A. galli* as well, and therefore,

although on March 28 no parasites were found in the pigeons that had been infected with *A. galli*, the possibility is not excluded that young squabs may be susceptible to infection by *A. galli*.

In the examination of the control chicks infected with *A. galli*, some interesting results were noted. The first chick killed on March 26, yielded 11 larval ascaridias, and while according to Ackert (1) the average length for a 29-day *A. galli* is about 31 mm., these varied in length from less than a millimetre to 9 mm. It first appeared as though the larvae were not the result of the original infection but were due to an infective contamination from some outside source. However, as the birds were kept under strictly sanitary conditions, and as none of the control birds showed the infection, the larvae must have been hatched from the eggs fed on February 26. The other chick infected at the same time was killed on April 16, to permit the parasite to reach maturity and thus make identification possible. Two male ascaridias were recovered from this chick, and here also, there was a considerable difference in their sizes, one measuring 42 mm. in length and the other 30 mm. Although the ascaridias were identified as *A. galli* they were only about three fifths of the normal length and the spicules were not fully formed. This lack of development may be due to some form of immunity reaction set up by the chicks, that interferes with the normal growth of the parasites, which are affected in an unequal manner. These results are quite different from any published by other workers in reference to the life history of *A. galli*.

Experiment 2

Artificial Infection of Pigeons with Heterakis gallinae

On January 21, several hundred female heterakis were teased out on a plate of glass and incubated in a relative humidity of 97.5%. On January 31, a heavy growth of fungus was noticed on the eggs, and a subsequent examination showed that those eggs teased out of the uterus and exposed contained air bubbles and were dead. The eggs protected by the uterine wall however, were still alive, and when examined on February 8, contained well developed larvae. It would seem that the fungus growth is lethal to the unprotected egg. The eggs were washed off into a small Petri dish and stored in a solution of 1% formol.

Only one pigeon was infected in this experiment and on February 27 it was fed about 125 eggs. The infection was repeated on March 1, 4, 8, 11, 15, 18 and 22. At the same time, in order to check the infectivity of the eggs, a chick was removed from the brooder, infected, and isolated in a cage. On March 23, 24 hours after the last infection, the pigeon was killed and the gut contents examined. Although a very thorough examination was made, no heterakis of any stage were recovered, nor were any eggs found. To determine whether the eggs were infective the control chick was killed, and on examination it yielded four adult heterakis. While this is not a large percentage of infection, it does show that some of the eggs were infective. These

results suggest the conclusion that *Heterakis gallinae* is not infective to adult pigeons. The possibility that it may be transmitted to young squabs, however, should not be overlooked.

What happens to the eggs when they enter the pigeon is an interesting point, and when we take into consideration the fact that no larvae or even eggs were found in the bird, the most likely explanation seems to be that the eggs hatch and the larvae, finding their environment incompatible, die and are digested by the host. There is the possibility that the eggs do not hatch at all and are passed out with the feces within 24 hours, or that the eggs do hatch and the larvae, not finding any caeca, pass on out. It seems unlikely however that they should completely pass through the alimentary canal in 24 hours. Moreover, as no larvae were ever found in the feces, the last explanation is unsatisfactory.

Experiment 3

Artificial Infection of Fowl with Ascaridia columbae

On December 20, the first attempt was made to incubate *Ascaridia columbae* eggs; they were placed in a shallow solution of 1% potassium dichromate. When examined on December 31, most of them contained active larvae. Again on January 15, eggs were sedimented on a plate of glass and sealed in a jar in 100% humidity. By January 25, although a fungus growth had appeared on the eggs, they nearly all contained active larvae. While the larvae appeared well developed, they were probably not infective. On February 23, eggs were sedimented on a plate of glass in a relative humidity of 90%. On February 25, another group of eggs was cultured in 1% solution of formol. This culture seemed to progress more rapidly, and although started two days later, reached the vermiform stage sooner than did the first mentioned culture. Eggs from the culture of February 25 were used in this experiment.

On March 18, ten chicks were each fed 1000 embryonated *Ascaridia columbae* eggs. On March 28, ten days after infection, one of the infected birds was killed, and a careful examination of the intestinal contents failed to reveal any ascaridia larvae. In order to find what happened to the *Ascaridia columbae* eggs after being swallowed by the chicks, the chicks were all re-infected on March 30, and individuals were killed and examined at 8, 24, 48, 72, 96, 120 and 144 hours after infection. At the same time, the feces were examined for eggs and larvae at intervals from one to 46 hours after infection.

As the larvae are very small, great care was taken in the examination of the intestinal contents of the birds. The gut was divided into its different parts—oesophagus, stomach, duodenum, small intestine, caeca and large intestine—and each part was opened up and individually shaken in water. The liquid was poured off and allowed to settle, while the mucosa was carefully examined for nodules or haemorrhagic spots. Any suspicious looking areas were scraped on to a compressorium and examined microscopically.

The liquid was allowed to settle for a considerable length of time, the upper fluid was poured into another vessel and allowed to settle in turn. This was repeated three or four times to make sure that no larvae were poured off with the upper fluid. The residue in each case was carefully examined under a binocular microscope. When larvae were found they were transferred to a slide, fixed by passing the slide over the flame of a spirit lamp, and studied under a microscope. The caeca of the chicks infected for more than 72 hours were not examined, and only in the eight-hour infection was any part above the duodenum examined.

In the chick killed eight hours after infection, eggs were recovered in the crop and the stomach. No larvae were found in any part of the gut.

The chick killed 24 hours after infection had numerous larvae in the small intestine; none was found in any other part of the gut, or in the immediate feces. Apparently the eggs hatch somewhere between eight and 24 hours after infection.

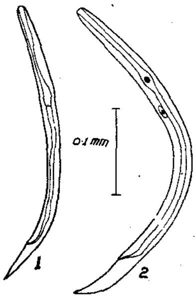
DESCRIPTION OF THE LARVAE (FIGS. 1, 2)

Relatively thick-bodied larvae; parallel sided body becoming somewhat thinner posteriorly, with a pointed tail; length 0.34 mm., width at widest part, distal portion of oesophagus, 0.018 mm.; oesophagus 0.12 mm. long or a little more than one-third body length, distal portion slightly expanded; intestine narrow, leading to an anus with slightly prominent lips, 0.049 mm. from tip of tail; the interior of the body filled with granular material.

The chick killed 48 hours after infection yielded five larvae from the small intestine. Here again, no larvae were found in any other part of the gut or in the immediate feces. The length in this case was 0.354 mm., the oesophagus 0.126 mm. and the distance from the tip of the tail to the anus the same as in the 24-hour larvae. These larvae were similar to the 24-hour one in all other respects except that here an excretory pore and duct could be made out 0.088 mm. from the anterior tip of the body.

The chick killed 72 hours after infection harbored five larvae in the posterior part of the gut. The larvae in this case were very similar to those obtained from the chick infected 48 hours, but were slightly longer.

The chick killed 96 hours after infection had four larvae in the posterior part of the small intestine and one in the large intestine. The larvae were approximately 0.39 mm. long, and were proportionately broader than the 24-hour larvae, the width being 0.023 mm. The oesophagus had a shorter and more distinct posterior bulb but was practically the same length. A short distance behind the bulb of the oesophagus was what appeared to be a genital anlage. The distance from the anus to the tip of the tail was 0.06 mm. (Fig. 2).



FIGS. 1 AND 2. *Ascaridia columbae, larval stages.* FIG. 1. *24 hours after infection.* FIG. 2. *96 hours after infection.*

No larvae were detected in the chicks killed 120 and 144 hours after infection.

The examination of the feces failed to reveal any eggs or larvae before 24 hours after infection. From 24 to 34 hours, larvae about 0.34 mm. long were recovered. No eggs were ever found, nor were any larvae found after 34 hours.

It would appear, therefore, that the eggs of *Ascaridia columbae* hatch in the chick between 8 and 24 hours after infection, and from 24 to 34 hours the great proportion of them pass out with the feces. However, some remain in the small intestine until between 96 and 120 hours after infection, when they die and presumably pass out with the feces or are digested by the host.

Acknowledgments

This iuvestigation was carried out under the supervision of the Director of the Institute of Parasitology. It is with pleasure that I express my thanks to the Director and the staff for their invaluable advice and assistance.

I would also like to express my appreciation to Messrs. Henry Gatehouse and Son for supplying pigeon material and to Mr. Louis Meyer for supplying the living pigeons used during the investigation.

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A NOTE ON THE REDUCTION IN THE FECUNDITY OF *TRIBOLIUM CONFUSUM* DUE TO EATING ITS OWN EGGS[1]

By John Stanley[2]

Some time ago (1) the writer showed that the growth of the egg population of *Tribolium confusum* could be described by an expression

$$T = \frac{c}{a} N_2 + \frac{ad - bc}{a^2} \log_e \left(\frac{aN_2 + b}{b} \right)$$

and this curve was figured in (1).

On making an attempt to produce this curve experimentally by removing the old eggs every four days and replacing them by an equivalent number of new young eggs, a curve of roughly similar form was obtained for the early stages, with evidence of a later periodic fluctuation instead of an approach to an asymptote.

Further experimental work, as well as mathematical analysis of the situation, shows that the curve actually obtained is not nearly as steep as it should be if the egg-laying rate remains constant, and there is good evidence to show that the eating of eggs reduces fecundity at a later time. This effect can be shown to be competent not only to flatten the curve but also to produce the periodic fluctuations that occur. A full description of this matter will be made in a later publication.

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[1] *Manuscript received February 22, 1937.*
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RAPHIDASCARIS LAURENTIANUS SP. N. (ASCAROIDEA) FROM *SALVELINUS FONTINALIS* (MITCHILL) IN QUEBEC[1]

By L. R. Richardson[2]

Abstract

A new Raphidascarid nematode, *Raphidascaris laurentianus* is described from material collected from the intestine of the speckled trout. The specimens were obtained from Labelle County, Quebec. *R. laurentianus* is of moderate size and is characterized by having delicate caudal alae, parallel uteri, and only five to twelve pairs of preanal papillae. This is the first definite record of such a nematode from speckled trout.

The records of nematodes parasitic in the speckled trout (*Salvelinus fontinalis* (Mitchill)) in eastern North America have been few. Within recent years several new species have been recorded. Skinker (3) has described *Metabronema canadense* from a single specimen obtained from a trout taken at the Matamek River, Quebec. Chandler (1) has described *Cystidicoloides harwoodi* from specimens taken from the stomach of trout collected near Elizabethtown, northern New York state, while Simon and Simon (2) have described *Philonema agubernaculum* from speckled trout and other hosts in the Wyoming area.

An examination of the intestines of six speckled trout collected at Lac Sept Frères, Labelle County, Quebec, in June of 1933, during the course of a biological survey conducted by the Department of Public Works, Game and Fisheries, showed the presence of large numbers of a new Rhapidascarid nematode. The worms were present throughout the length of the intestine with the exception of the rectum and the stomach. As many as 75 worms, ranging in length from 5.0 mm. to 29.0 mm. and representing both sexes, were taken from the gut of a single trout. The majority of the worms were present in the pyloric caeca. The largest females were collected from the lower portion of the intestine. Immature specimens were common and ranged in length up to 10.0 mm.

The hosts were also heavily infected with the trout tapeworm (*Eubothrium salvelini* (Schrank, 1786)) and with the small coronate trematode *Crepidostomum fausti* Hunninen and Hunter 1933.

The present specimens are located within the genus *Rhapidascaris* on the basis of the arrangement of the alimentary canal and associated structures. The presence of a ventriculus and ventricular appendix, and the absence of other caeca, the cuticular expansions of the lips and absence of dentigerous ridges, are characters descriptive of this worm and diagnostic for this genus. The presence of caudal alae does not seem to have been noted in other worms of the genus and may serve to set aside the present specimens from previously described species in the genus.

[1] *Manuscript received March 12, 1937.*
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[2] *Assistant, Department of Zoology, McGill University.*

Raphidascaris laurentianus SP. NOV.

In the present collection the females reach a maximum length of 29.0 mm. with a maximum diameter of 1.08 mm. at the beginning of the posterior third of the body, and a diameter of 0.72 mm. at the level of the vulva. The average female specimens are from 17.0 mm. to 25.0 mm. in length. In these the posterior portion of the body is slightly wider than the anterior, and the diameter of the body increases gradually from the narrow head back to the level of the vulva where it varies from 0.52 mm. in the smaller specimens to 0.70 mm. in the larger. Behind this point, the body is of practically uniform diameter nearly to the level of the anus. With the development of large numbers of eggs in the uteri and median vagina, the posterior portion of the body becomes obviously swollen. The tail is long, sharply pointed and tapering gradually from just before the anus to extend 0.5 mm. beyond that point. The cuticle is striated, there being five to seven striations in a length of 0.1 mm. The cuticular ridges frequently present a retrorse appearance. Symmetrical lateral cephalic alae are present. These are narrow, 0.12 mm. in breadth at their widest point, and extend backwards from the neck to a point just beyond the

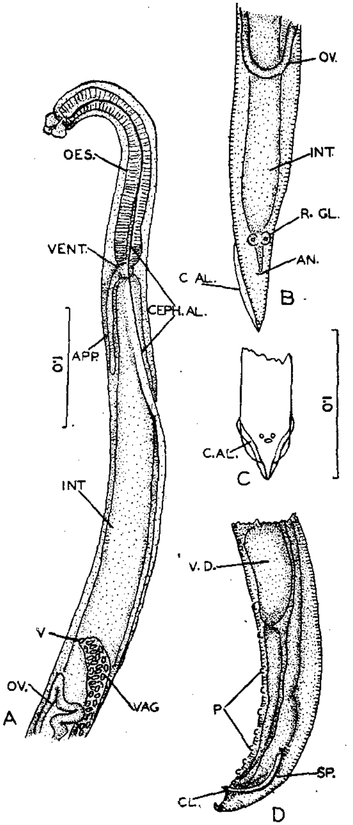

FIG. 1. *A. Anterior third of adult female, 20.0 mm. long, drawn in ventro-lateral view. B. Posterior end of adult female in ventro-lateral view. C. Outline view of posterior end of male drawn from the ventral aspect. D. Posterior end of male, lateral view. All scales in mm.* AN., *anus;* APP., *ventricular appendix;* C.AL., *caudal alae;* CEPH. AL., *cephalic alae;* CL., *cloaca;* INT., *intestine;* OV., *ovary;* P., *papillae;* R. GL., *rectal glands;* SP., *spicule;* VAG., *vagina;* V.D., *vas deferens;* VENT., *ventriculus;* VULV., *vulva.*

vulva. Delicate short caudal alae are present and difficult to detect. They extend from a point 0.5 mm. before the anus to the tip of the tail. The caudal alae are 0.030 mm. in width. There are no sulci on either the cephalic or the caudal alae. There are three lips, one dorsal and two subventral. The dorsal lip has two papillae, the ventral lips one each. The border of each lip is emarginate. The oesophagus in the female is 2.0 mm. to 3.4 mm. in length and averages $\frac{1}{8}$ of the total length of the body. The ventriculus is 0.15 mm. to 0.17 mm. in length and is wider than long. The thin-walled ventricular appendix is 0.9 mm. to 1.1 mm. in length. The gut is simple, expanded throughout its length and is joined to the anus by a short, narrow tube with delicate walls. There is no intestinal caecum. The vulva is situated at a point distant $\frac{1}{3}$ the total length from the anterior end of the body. The vulva is not protected by prominent lips. The median sac-like vagina is elongate and terminates posteriorly in two simple uteri. The latter are parallel, not divergent, and pass straight backwards to a point 1.5 mm. from the anus where they turn forwards into the oviducts. The latter pass forward nearly to, but not reaching the level of the vulva and then return as the ovaries. The ovaries are greatly convoluted with the exception of the posterior portion, which terminates before reaching the anus. The ovaries are lateral to and conceal the other portions of the reproductive system. The ova removed from a gravid female are subspherical and have a diameter of 0.080 mm.

The male is smaller than the female and reaches a maximum length of 23.6 mm. with a diameter of 0.78 mm. The majority of the mature males in the present collection are 17.0 mm. to 22.0 mm. in length. The body is of an almost uniform diameter posterior to the beginning of the second third of its length, but tapers gradually forwards from this point to the narrow head. The posterior end of the body is bent ventrally into a semicircle. The body terminates more abruptly than in the female, posterior to the cloaca the short tail taking the form of a small high cone. The mouth parts are similar to those of the female. The cuticle is striated. Symmetrical lateral cephalic and caudal alae are present and similar to the same structures in the female. The cephalic alae extend to the level of the anterior coils of the testis. The cuticle bears five to twelve pairs of low, non-pedunculate preanal papillae and one pair of adanal papillae. The number increases in the larger males, some of which also have two or three pairs of incompletely developed papillae anterior to the normal ones. The oesophagus is $\frac{1}{6}$ to $\frac{1}{10}$ the total length of the body. The central canal is corrugated. The ventriculus has a longitudinal diameter $\frac{1}{12}$ that of the length of the oesophagus. The appendix and gut are the same as in the female. The testis is convoluted and reaches forward to the posterior end of the ventricular appendix. The coils of the testis are wider than long, close and more regular than the coils of the ovary in the female. The testis terminates in a median, elongate vesicula seminalis. The spicules are equal, curved and 0.45 mm. to 0.70 mm. in length. Each

spicule bears a wing in the form of a gutter. The shaft of the spicule is 0.016 mm. in diameter, the wing 0.044 mm. in width. The spicules terminate with an obtusely pointed distal end.

The present specimens are distinct from *R. brachyurus* Ward and Magath, 1916 (5) in the presence of papillae on the tail of the male. They differ from *R. cayugensis* Wigdor, 1918 (6) in having the uteri parallel and not divergent at their entry into the median vagina. *R. laurentianus* is distinct from *R. canadensis* Smedley, 1933 (4) in having the cephalic alae equal, and lacking a sulcus; a smaller number of papillae on the caudal end of the body in the mature male, a more posterior position of the vulva in the female and in having eggs of a larger size. The present specimens are smaller worms than *R. acus* (Block, 1779).

I have examined the viscera of several northern pike (*Esox lucius* Linn.) taken from lakes in Labelle County, in the expectation of obtaining specimens of *R. canadensis* for closer comparison with the present specimens, but so far without result. These pike were all infected with *Proteocephalus pinguis* La Rue 1911. Specimens of pickerel (*Esox niger* LeSueur) from Brome Lake, Brome County, were heavily infected with a Raphidascarid nematode the larvae of which are commonly found encysted in the liver of perch at this lake. These specimens are also distinct from any described Raphidascarid.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 15, SEC. D. JUNE, 1937 NUMBER 6

THE PHYSIOLOGY OF THE SHEEP TAPEWORM, *MONIEZIA EXPANSA* BLANCHARD[1]

BY ROBERT ARNOLD WARDLE[2]

Abstract

The influence of saline media upon the longevity, water content and polysaccharide content of *Moniezia expansa* is discussed. Worms may live two or three days in balanced salines but in the majority of experiments they die between 9 and 12 hours. Addition of glucose abbreviates the longevity period in saline media. The water content is affected only slightly by balanced salines but is influenced by the concentration of sodium chloride and by the presence of sugars and amino acids; behavior in saline media cannot be correlated wholly with changes in water content. The polysaccharide content is not significantly different from mammalian glycogen, constitutes 0.35 to 5.25% of the fresh weight, remains stable during immersion of the living worm for six hours in media that reduce muscle tonus, but decreases during immersion in media that encourage muscle tonus. Glycogen increase occurs when the medium contains glucose up to 1%, but not when the glucose content exceeds this amount or when the glucose is replaced by other sugars, by amino acids, or by glycoproteins. Saline media are adjudged unfavorable to tapeworm existence *in vitro*.

1. Introduction

The literature on the subject of tapeworm physiology is scanty and the neglect of this aspect of comparative physiology can only be attributed to the lack of established technique for keeping a tapeworm alive outside its host for a sufficient length of time to permit of physiological experimentation and observation. So closely adapted is an adult tapeworm to its habitat—which, with few exceptions, is the small intestine of some chordate animal—that its life in the relatively crude laboratory imitations of this habitat is always brief, the animal disintegrating with rapidity whether in sterile or in oligoseptic media.

The data presented below are the results of a series of experiments extending over three years with a typical tapeworm *Moniezia expansa*, employing the saline nutrient media commonly used for animal cultivation experiments, and taking as criteria of the favorableness of such media their influence upon the longevity of the tapeworm, upon its water content, and upon its polysaccharide content.

2. Material and Methods

The sheep tapeworm, *Moniezia expansa* (Rudolphi, 1810) Blanchard, 1891, was selected as a subject for this study because it is abundant and easy to obtain during the late summer in Canada, and because it has been used by

[1] *Manuscript received April 26, 1937.*
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several other workers in this field, notably by Brand, and Cook and Sharman, the latter workers referring to their material erroneously as "*Moniezia trisonophora*". It is, however, not an ideal tapeworm for experiments of this nature since it cannot be obtained in quantity during the winter and spring, and is somewhat sensitive to removal from the host.

The tapeworms can be obtained most readily and quickly by hand-squeezing the lamb gut slowly from the pylorus to the beginning of the large intestine, and although each lamb may contain only 4–10 worms, the large scale on which lambs are slaughtered in the Canadian packing industry, and the high incidence of this worm in western Canadian lambs, permit the collection of several hundred worms per hour. Medium sized specimens, 30–50 cm. in length, that show activity and are unstained by bile, should be selected, rinsed in physiological saline, drained on soft cotton cloth, and placed as soon as possible in the experimental media. If kept at 20° C. in moist cotton cloth, however, they will live with negligible muscular activity and negligible polysaccharide and water loss up to 12 hours.

The media used were unsterilized but freshly prepared saline solutions alone or plus the addition of 1% of some nutrient substance,—sugars, amino acids, glycoproteins. Four saline bases were used in most of the experiments:

1. *Tyrodes solution*, used in single strength and two and a half times single strength; the stronger solution was used on the assumption, that later proved erroneous, that it would be nearer isotonicity for the worms than would the ordinary concentration.

2. *Phosphate saline*, which was Tyrodes solution with the sodium acid phosphate content increased from 0.02 to 0.2%, on the suggestion of Magee and Reid (10) that the presence of 0.2% of sodium acid phosphate stimulates the absorption of glucose by the intestinal mucosa of the rat.

3. *Bile saline*, a solution of 0.8% sodium chloride in water plus the addition of 10% fresh sheep bile, this concentration of bile being assumed to resemble the concentration present in the small intestine of the lamb.

4. *Sodium glycocholate*, in 1% aqueous solution, used as a ready solvent for certain nutrients (lecithin, cholesterol) not soluble in the other bases.

In the water content experiments, tests were made also with distilled water, half, double, triple, quadruple and quintuple strength Tyrodes solution; with a Ringer solution formula recommended by Brand (4); and with another Ringer solution formula recommended by Cook and Sharman (6); and with $M/5$ and $M/10$ sodium chloride solutions. These media were used in 200-ml. quantities in 250-ml. Erlenmeyer type Pyrex flasks kept in a dry incubator at 36-38° C. for a period of six hours only; five worms were used in each test and the test was repeated at least three times.

It may be noted that, throughout this paper, the term *fresh weight* means the weight of freshly collected, living material, after rinsing in physiological saline and draining on soft cotton cloth until the surface is barely moist;

the term *alcoholic weight* means the weight of tapeworm material after storage for one month in 95% ethanol, and subsequent drainage until superficially dry; and *dry weight* means the weight of the worm material after desiccation to constant weight, usually for 4–5 hr. at 150° C., care being taken to avoid charring. Among 100 specimens, the alcoholic weight was found to be 50.1% of the fresh weight, the dry weight was 10.1% of the fresh weight.

3. Tapeworm Longevity

Very little accurate information is available concerning the longevity of tapeworms whether *in situ* or *in vitro*. The author (18) has summarized previously such information as was available up to 1933, and can add to that summary merely the observation by Seddon (13) that in Australian lambs *Moniezia expansa* lives not longer than 65–70 days; the observation by Gordon (8) that in Australian lambs *Moniezia expansa* produces eggs for at least 350 days; and the observations of Leiper (9) of the persistence of *Diphyllobothrium latum* in man during five years and of *Diphyllobothrium mansoni* in dogs for eight years.

The difficulty in observations of tapeworm longevity *in situ* is that of eliminating the possibility of host re-infestation, a difficulty that has been stressed by Ward (16) in a critical analysis of a large number of cases of reputed longevity in *Diphyllobothrium latum*.

The difficulty in observations of tapeworm longevity *in vitro* is that of deciding whether the tapeworms are alive at all, and of deciding the exact moment of death. Absolute values of tapeworm longevity *in vitro* are of little value as physiological data since for part of the time the animal may be moribund or disintegrating. Nor can it be assumed, as was done by Cook and Sharman (6) that carbon dioxide production is indicative of tapeworm vitality, since the experiments of Brand and Weise (5) suggest that carbon dioxide production by tapeworms is a by-product of glycogenolysis, and not a by-product of aerobic respiration, and it is possible that such glycogenolysis is as much a post-mortem as a pre-mortem phenomenon. In the light of Brand's results, the belief of Cook and Sharman that *Moniezia expansa* will live 200 hours in their saline solution plus $M/10{,}000$ sodium hydroxide needs confirmation.

The author (17, 18) has attempted to show in previous papers that tapeworms *in vitro* pass through a period of undulant activity, sometimes brief, sometimes prolonged, before passing eventually into either a condition of flaccidity or a condition of tetanic contraction, both irreversible, and has argued that the termination of longevity should be taken arbitrarily as the moment when undulant activity ceases.

A series of experiments was carried out to determine the duration of undulant activity of *Moniezia expansa* in Tyrode's solution, Brand's solution, Cook and Sharman's solution, $M/5$ sodium chloride and $M/10$ sodium chloride. These salines were used alone, and again with glucose added in amounts

equivalent to $M/5$, $M/10$, $M/100$, $M/500$ and $M/1000$. One set of experiments was made with sterilized media, one set with unsterilized but freshly prepared media. Each experiment was repeated five times, five worms being used each time under the conditions of temperature given above. Observations were made at hourly intervals.

The results obtained were extremely unsatisfactory. The moment of transition from activity to passivity is difficult to determine among a number of intertwined worms and there is considerable variation between individuals. The general findings were as follows:—

1. In glucose-free media or media with $M/500$ or less of glucose, the activity period varied from 24–72 hr. in Brand's solution, 24–48 hr. in Tyrode's solution, 9–12 hr. in Cook and Sharman's solution, 9–12 hr. in $M/5$ and $M/10$ sodium chloride.

2. In media containing glucose exceeding $M/500$ in amount, the period of undulant activity was always short and varied from 9–12 hr.

In none of the media that were tested, whether sterile or non-sterile, whether glucose-free or glucose-containing, at hydrogen ion concentrations between 6.0 and 9.0, could specimens of *Moniezia expansa*—only one hour distant from life *in situ*—be relied upon to remain undulant longer than 12 hr. although occasional specimens might remain so for two or three days. The fact seems to be that no two individuals of a tapeworm species will react in the same way to the adverse conditions of an artificial environment.

Taking undulant activity as a criterion of the favorableness of an artificial medium to *Moniezia expansa*, fluid saline media are unfavorable to its life *in vitro*.

No reason for the brevity of the undulant activity period in saline media can be given. The duration of the experiment was too short for such inhibition to be caused by bacterial toxins or by the by-products of metabolism—carbon dioxide, succinic acid, lactic acid. Stoppage of activity in a sugar-free medium might be suspected to arise from glycogen exhaustion were it not for the fact that, as shown in later experiments, the glycogen content is not rapidly exhausted by starvation, and in fact is considerably increased by the presence of glucose in the medium.

4. Water Content

The author (17, 18) has discussed in previous papers the change of form induced in living tapeworms by immersion in media that are hypotonic and hypertonic in the physiological sense; how tapeworms tend to expand and become flaccid in hypotonic salines, and to become increasingly foreshortened and contracted in media whose electrolyte content is gradually increased from isotonicity.

That such morphic changes are associated with changes in water content might seem to be suggested by the high water content of tapeworms (usually between 85 and 90%), by the absence from tapeworms of exoskeletal and endoskeletal structures, and by the admitted porosity of the tapeworm surface.

Experimental confirmation of this suggestion is illustrated by Table I, which indicates the mass per original gram of living *Moniezia expansa* after immersion for six hours at 36–38° C. in various aqueous media.

TABLE I

MASS PER ORIGINAL GRAM OF LIVING *Moniezia expansa* AFTER SIX HOURS' IMMERSION AT 36–38° C.

		Arabinose	Glucose	Galactose	Levulose	Maltose	Glycine	Mucin
Water	3.3							
Tyrode × ½	2.0							
Tyrode × 1	1.004	1.16	1.15	1.04	1.04	1.15	1.87	1.28
Tyrode × 2	0.70							
Tyrode × 2½	0.88	0.53	0.71	0.38	0.67	0.50	0.52	0.56
Tyrode × 3	0.88							
Tyrode × 4	0.76							
Tyrode × 5	0.61							
Sod. glycol	0.83	0.88	0.96	0.86	0.84	0.91	0.67	—
Bile saline	1.01	0.82	0.98	0.60	0.86	0.82	0.74	1.33
	Glucose, %							
	—	0.1	0.5	1.0	2.0	5.0	10.0	20.0
Phosphate saline	0.99	1.09	0.94	0.82	0.50	0.38	0.38	

Selected whole worms were used. They were rinsed, drained on soft cotton cloth, weighed, immersed in the medium for six hours, then removed, rinsed, drained, and weighed again. The media used are named in the table.

Any change in weight induced in a tapeworm by brief immersion in a fluid must be due to water loss or water gain, since the glycogen or fat changes during six hours represent extremely minute fractions of the worm's weight. The data presented in Table I suggest that least disturbance of the initial water content is induced by Tyrode's solution, phosphate saline, and bile saline, that is to say, by those media that are isotonic with mammalian tissues. In hypotonic media such as water and half-Tyrode, there is absorption of water which may account to some extent for the flaccidity of tapeworms in such solutions. As the sodium chloride content of the medium is increased, the tapeworm shows increasing water loss until, when concentrations greater than quadruple strength are used, the tapeworm is definitely losing water and contracting visibly.

The influence of added nutrients upon water content seems to vary according to the saline base, but glucose in amounts greater than 1% seems to induce water loss.

The relatively slight disturbance of the tapeworm water content by isotonic saline media would suggest that on this criterion such media are suitable for tapeworm cultivation *in vitro*. Since, however, in such media the tapeworm is far more active and more extended than it is when *in situ*, muscular activity must to some extent be influenced by penetration of ions or molecules from the medium, a conclusion arrived at previously by the author (18), from experiments with *Nybelinia surmenicola*.

5. Polysaccharide Content

Appreciable quantities of a polysaccharide substance have been shown to occur in such gut-frequenting helminths as have been analyzed, and in the germinal membrane of the vesicular larvae of taeniid tapeworms. Reference should be made to Brand (3, 4, 5) and to Schopfer (12) for a résumé of the existing literature on the question.

Among 30 specimens of *Moniezia expansa* analyzed by Brand (4) the polysaccharide content, expressed in terms of reducing sugar to bring the figures into line with those presented below, and assuming that Brand used Pflüger's factor of 0.927, ranged from 0.99 to 5.35%, with a mean value of 2.69% of the fresh weight. This polysaccharide content may be isolated by the method of Good, Kramer and Somogyi (7) and its reducing sugar content estimated iodometrically by the method of Shaffer and Hartmann (14); or it may be further purified by the method of Somogyi (15) and dried over calcium chloride. Thus prepared from *Moniezia expansa*, the polysaccharide constituent is an amorphous white powder, readily soluble in water, beginning to char at 210° C. and attaining maximum charring at 245° C. When hydrolyzed by heating for three hours in 3% hydrochloric acid at 90° C., it yields a reducing sugar content of 95.27%: the nature of the residue was not determined. The reducing sugar obtained answers all the recognized criteria of *d*-glucose, and the polysaccharide appears therefore not significantly different from mammalian glycogen.

Among 16 selected worms, analyzed within one hour of removal from the host, the polysaccharide content expressed in terms of reducing sugar, and using the conversion factor of 0.961 suggested by Bell and Young (1), ranged from 0.35 to 5.23% of the fresh weight, with a mean value of 3.14%. Among 17 worms analyzed after one month of storage in 95% ethyl alcohol, the polysaccharide content similarly expressed ranged from 0.52 to 4.89% of the fresh weight, with a mean value of 3.31%. Among 400 thirds of *Moniezia* individuals, after one month of storage in ethyl alcohol, the polysaccharide content ranged from 1.55 to 3.96% of the fresh weight, with a mean value of 3.22%. Ethyl alcohol therefore, as could be expected, inhibits glycogenolysis and is a convenient storage fluid for material whose polysaccharide content is to be estimated. On the other hand, among 10 specimens stored for one month in 10% formalin, the polysaccharide content was only 0.19% of the fresh weight, so that formalin-preserved tapeworms are useless for glycogen determination.

The polysaccharide content of *Moniezia expansa*, therefore, is relatively high, since it constitutes approximately 3.2% of the fresh weight, or approximately 32% of the dry weight and may exceed the fat content or the protein content. Microscopical examination of sectioned *Moniezia* material stained by the glycogen demonstration method of Best (2) shows the polysaccharide content to be distributed uniformly throughout the parenchyma but to be absent from the cuticle, dermis, longitudinal and circular muscles, uterine reticulum and eggs. Its absence from the muscles of worms killed by plunging

into boiling ethyl alcohol, suggests a definite correlation between polysaccharide content and muscular activity.

It is generally conceded by students of helminth physiology that the polysaccharide constituent of a parasitic helminth is synthesized from glucose withdrawn from the host fluids, similar synthesis from amino-acids being precluded by the lack of aerobic respiration, and that in the case of tapeworms such host glucose must enter through the cuticle and be synthesized into glycogen in the parenchymal cells. Ortner-Schönbach (11) suggests that the glycogen-gorged parenchyma acts as a source of glucose for the muscles, dermis and gonad-ducts where it is degraded, and as a source of sugar for the ovaries, vitellaria and gland cells where it is re-synthesized into glycogen. Admission must be made, however, that the hypothesis of glycogenesis from environmental glucose has received little experimental support. Ortner-Schönbach could detect no histological evidence of alterations in glycogen content between control specimens of the horse tapeworm *Anoplocephala* and specimens that had been kept for seven days in saline solutions and in saline-glucose solutions. Apart from the possibility that the experimental worms may not have been alive during the whole of the seven days, the present histological methods of demonstrating tissue glycogen are far from being sufficiently precise to indicate differences in glycogen concentration.

Brand (4) obtained inconclusive results by the anaerobic immersion of specimens of *Moniezia expansa* in Ringer-glucose solution. In four experiments, one worm showed a glycogen gain of 0.13% after six hours' immersion at 37.5° C. but the other three showed glycogen losses of 0.02, 0.03, and 0.18%. In the opinion of the present writer the experimental error in experiments of this nature may be as high as 1%, that is to say, two halves of the one worm may differ in glycogen content to that extent, so that Brand's results merely suggest that *no* glycogen change occurs when *Moniezia expansa* is immersed for six hours at 37.5° C. in an anaerobic solution of Ringer-glucose.

More suggestive are Brand's observations that eight specimens of *Taenia hydatigena*, from a dog that had received a high carbohydrate diet, had a glycogen content of 8.53% of the fresh weight, whereas specimens from a dog fed on an ordinary diet showed a percentage of 4.99%, but even these figures cannot be seriously considered unless it be shown that the difference, 3.39%, is greater than the maximum variation that occurs normally in glycogen content between individuals of this tapeworm species.

The data presented in Table II are based upon six-hour immersion experiments with *Moniezia expansa* in various media at temperatures of 36–38° C. In such experiments advantage may be taken of the fact that fractionated tapeworms live just as long as intact ones. Each experiment made use of five selected living worms; each was cut into approximate thirds; one set was fixed in boiling ethyl alcohol and stored in ethyl alcohol for one month before being analyzed for initial glycogen content; a second set of thirds was im-

TABLE II

INFLUENCE UPON GLYCOGEN (G) CONTENT, EXPRESSED IN MILLIGRAMS OF REDUCING SUGAR PER GRAM OF FRESH WEIGHT, OF IMMERSION OF LIVING *Moniezia* FOR SIX HOURS AT 36–38° C. IN VARIOUS MEDIA

Medium	Initial G	Final G	Medium	Initial G	Final G
Dist. water	27.32	30.42	Tyrode × 1, plus		
Tyrode × ½	29.2	28.4	Arabinose	29.8	15.3
Tyrode × 1	23.56	22.16	Glucose	30.4	55.3
Tyrode × 2	30.1	30.4	Galactose	30.0	19.0
Tyrode × 2½	35.5	36.1	Levulose	37.6	16.2
Tyrode × 3	25.84	21.25	Maltose	32.7	14.3
Tyrode × 4	31.42	25.13	Glucosamine	20.9	21.4
Tyrode × 5	38.8	20.18	Glycine	26.7	22.5
Sod. glycol.	29.7	14.7	Tyrosine	31.6	38.14
Bile saline	34.4	19.4	Mucin	40.5	18.9
Phosph. sal.	33.2	32.3	Bile 10%	20.0	17.8
Tyrode × 2½ plus			Bile saline, plus		
Arabinose	20.07	10.15	Arabinose	28.86	5.8
Glucose	31.6	39.87	Glucose	26.62	41.4
Galactose	33.5	27.5	Galactose	25.08	14.29
Levulose	29.12	27.72	Levulose	38.8	14.08
Maltose	37.00	22.5	Maltose	31.07	7.09
Glycine	30.3	11.3	Nutrient agar	30.2	5.6
Glucosamine	36.4	34.8	Glucosamine	47.16	35.7
Mucin	24.3	25.1	Mucin	36.6	37.8
Sodium glycocholate, plus			Phosphate saline plus		
Arabinose	34.8	31.0	Glucose, 0.1%	39.3	28.6
Glucose	32.68	37.48	Glucose, 0.5	21.4	52.1
Galactose	21.9	21.6	Glucose, 1.0	41.2	27.0
Levulose	36.8	11.7	Glucose, 2.0	31.5	0.93
Maltose	39.8	13.08	Glucose, 5.0	32.05	1.40
Glycine	38.36	33.8	Glucose, 10.0	41.6	6.8
Tyrosine	38.34	31.2	Glucose, 20.0	41.2	7.8
Mucin	28.06	26.0			
Cholesterol	36.06	4.06			
Lecithin	24.2	4.07			

mersed in the saline base for six hours, then alcoholized and stored; the remaining set of thirds was immersed for six hours in the saline base plus the nutrient, and then alcoholized and stored. Each experiment was repeated three times.

In the table the polysaccharide contents are expressed in terms of milligrams of reducing sugar per gram of fresh weight, allowance being made for water content changes induced by the media.

The differences between pre-experimental and post-experimental polysaccharide contents are of significance only where they exceed the maximum variation that may occur in polysaccharide content between successive thirds of a group of five worms. To arrive at this, four groups of five trisected worms were taken and each set of thirds analyzed after one month in alcohol storage. For each of the four groups, the maximum differences in polysaccharide content between sets of thirds were 2.0, 4.2, 6.0 and 10.0 milligrams of reducing sugar per gram of fresh weight.

In the table, therefore, differences of less than 10 mg. between pre-experimental and post-experimental data must be discarded as without significance.

It may be assumed, from the data in Table I, that when living specimens of *Moniezia expansa* are immersed in saline media for six hours at 36–38° C., under aerobic conditions that:

(1) There are no significant changes in polysaccharide content induced by immersion in distilled water, half Tyrode, full Tyrode, double, two and a half, triple, quadruple Tyrode, and in phosphate saline; that is to say, in media in which the longitudinal musculature of the tapeworm, as compared with the longitudinal musculature of the worm *in situ*, is relaxed;

(2) There is polysaccharide loss induced by immersion in quintuple Tyrode, in 1% sodium glycocholate solution, and in bile saline; that is to say, in those media in which contraction of the worm occurs or where it vigorously undulates. Tempting as it is to assume that the polysaccharide loss represents consumption by the functioning musculature, it is not impossible that despite its large molecule, glycogen may pass out through the body surface with the abstracted water. Glycogen can be detected in the mucus covering the freshly collected worms in quantity as high as 1.3 mg. per gm. fresh weight. The loss of glycogen in bile saline does not fit in with either conception since the water content loss in bile saline is negligible, and the undulant activity is scarcely more vigorous than when the worm is in Tyrode solution;

(3) There is a gain in polysaccharide content when glucose is present in concentrations of 1% in Tyrode's solution, bile saline, and probably two and a half Tyrode, but in sodium glycocholate and phosphate saline the polysaccharide content in the presence of 1% glucose remains unchanged;

(4) No polysaccharide gain was obtained by the substitution for glucose of other sugars, of amino acids and of glycoproteins, nor, in phosphate saline, of higher concentrations of glucose.

The glycogen changes shown above do not altogether support the view of Ortner-Schönbach (11) that the primary function of tapeworm glycogen is the nourishment of the developing genitalia, but suggest rather that it is primarily a source of fuel for the longitudinal musculature when the latter is maintaining the condition of muscular tonus which characterizes the normal worm, and that it is depleted in any artificial medium in which this tonus is maintained or in which it is exaggerated into tetanic contraction, and depleted only very slowly or scarcely at all when the musculature relaxes completely or undergoes periodically repeated phases of relaxation as during undulant activity.

6. Conclusions

If maintenance of normal longevity, water content and polysaccharide content be accepted as criteria of the favorableness of artificial media to tapeworms, and if *Moniezia* be regarded as representative of tapeworms in general, the inescapable conclusion from the data presented above is that the saline media usually employed in physiological studies are useless for the study of tapeworm physiology, and that conclusions as to the nature of tapeworm metabolism that are based upon experiments carried out in such media cannot be accepted as giving an accurate picture of the physiological processes taking

place in the tapeworm that is lying in an animal gut. That is to say, the bulk of information already accumulated upon tapeworm physiology, scanty as it is, is practically worthless, and workers in this field of physiology will have to adopt a technique more akin to that of the bacteriologist than to that of the physiologist. Successful tapeworm cultivation *in vitro* may require that the animal be embedded in a semi-fluid gel after preliminary aseptic treatment. The technique of such asepsis has yet to be established, and the author is unaware of any experiments in this direction beyond his own crude and unsuccessful experiments with *Nybelinia surmenicola* (18). On the other hand, the nutritional requirements of such a tapeworm when *in vitro* may prove to be less exacting than has been supposed and it may not be necessary to duplicate the nutrient complex that surrounds the tapeworm *in situ*. Glucose seems definitely the one carbohydrate requirement of tapeworms; the amino acid requirement may be equally simple; there may be no fat requirement.

7. Acknowledgments

The author must express his thanks to Miss M. J. Gotschall and Mr. Herman Moore of the University of Manitoba, for much help with the tedious routine of tapeworm analysis, and to Canada Packers Limited of Winnipeg, for courteous and unrestricted facilities for obtaining material.

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Canadian Journal of Research

Issued by The National Research Council of Canada

VOL. 15, SEC. D. JULY, 1937 NUMBER 7

STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

IV. ON THE LETHAL EFFECTS OF SOME NITROGENOUS FERTILIZERS ON THE FREE-LIVING STAGES OF SCLEROSTOMES[1]

By I. W. Parnell[2]

Abstract

The effect of ten of the commoner nitrogenous fertilizers on the free-living stages of Sclerostomes, is discussed. Urea is the most lethal. Under the conditions of the experiments, which are otherwise ideal for the survival of the larvae, one part of urea to 125 parts of fresh horse feces is necessary to sterilize them. The proportions of the others tested are: Calurea, 1 : 80; powdered cyanamide, 1 : 50, with granular cyanamide slightly lower; potassium nitrate, sodium nitrate and Calnitro, 1 : 20; calcium nitrate and diammonium phosphate (dry or in medium strength solution) 1 : 17; Nitro-chalk, about 1 : 16 (but should not be used in strong solution); ammonium sulphate, 1 : 14.

When urine is not available to sterilize feces against the free-living stages of bursate nematodes, the next most economical group of chemicals to use will probably prove to be artificial fertilizers, since part, or all, of their value should be recovered in increased fertilizing value and increased crop yields.

This paper reports on the lethal values of ten of the more common nitrogenous artificial fertilizers, *viz.*,

Urea	Calnitro
Calurea	Calcium nitrate
Calcium cyanamide	Diammonium phosphate
Potassium nitrate	Nitro-chalk
Sodium nitrate	Ammonium sulphate

Tests with a few other nitrogenous chemicals will be described in subsequent papers: these will include calcium cyanide, ammonium carbonate, ammonium chloride, ammonium sulphide, ammonium nitrate and chloropicrin.

In addition to considerations of the relative cost of the fertilizers, of types of land and of crops for which the treated feces are to be used, the fact that some fertilizers or chemicals, when mixed with feces, caused loss of nitrogen as free ammonia must be remembered when a decision is being made as to which is the most economical sterilizing agent. Loss of nitrogen is most likely to occur through chemical reactions when alkaline fertilizers are mixed with manure; loss may also occur through bacterial action.

[1] *Manuscript received May 11, 1937.*
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All the fertilizers mentioned above were tested for loss of ammonia by mixing one part of the chemical with 10 parts of fresh horse feces, and incubating them under similar conditions to the other tests reported in this paper. The temperature was 25 ± 2° C. and it seems legitimate to assume that decomposition would be comparatively rapid. The fertilizers were examined for the presence of free ammonia after three and seven days. The greatest loss was found to occur with urea, Calurea, and cyanamide.

Few data are available on the chemical control of the free-living stages of parasitic nematodes, and of those many are not comparable with the present series of experiments, because the fertilizers were tested either on eggs or larvae without the presence of feces or, if tested in feces, the criterion was the absence or presence of colonies of larvae on the side of the culture jar. The first of these would not occur in practice while the second does not demonstrate the survival of a few larvae. However, the results obtained by Nöller and Schmid (7) and Enigk (2) with horse Sclerostomes, suggest that ammonium sulphate, sodium nitrate and calcium cyanamide, even in dilute solutions, do have some effect.

The work on the control of hookworm larvae with artificial fertilizers is more comparable. Large quantities of ammonium sulphate and Chili nitrate have been tested in China, at the rate of one part of fertilizer to three of night soil, and it has been shown that the larvae are quickly killed (1). In Italy, Penso (14), who has tested calcium cyanamide and other chemicals, recommends the use of calcium cyanamide for sterilizing human feces against hookworms, and also recommends (15) the addition of 12 kg. of the fertilizer to sterilize one cubic metre of manure against the Strongyles of domestic animals. Steverel and Berny (18) and de Giorgi (3), who used a 0.3% solution, agree that calcium cyanamide is lethal to Ancylostome eggs and larvae.

Newbigin and Morgan (5), in investigating chemicals for the control of the nematodes of poultry, found that 224 lb. of sulphate of ammonia per 120 sq. yd. was of no value in destroying non-bursate nematode eggs on poultry runs.

Artificial fertilizers have, however, been tested more frequently against the plant nematodes, *Anguillulina dipsaci*, *Heterodera schachtii* and *H marioni*. Unfortunately, the fertilizing effect of the chemicals on the plants has usually masked considerably the effect on the nematodes. This has made it difficult to compare the results on plant nematodes with those on Sclerostome larvae, while varied soil, climatic and other conditions tend to make the results contradictory. Nevertheless, some selected results may at least be suggestive to those interested in the control of the free-living stages of nematodes. Newton, Hastings and Bosher (6) have shown that urea, ammonium sulphate, potassium nitrate, ammonium carbonate and ammonium chloride in 2% solutions are not rapidly lethal to the bulb nematode. Hurst and Triffitt (4), found that, weight for weight, urea and calcium cyanamide were equally efficient against *H. schachtii*, but that on a nitrogenous basis, calcium cyanamide was the more effective. Small (17) failed to control

H. marioni on tomatoes with nine ounces of calcium cyanamide per square yard. Watson (20) states that good results can be obtained with half a ton of cyanamide per acre on sandy loam, and reports effective sterilization with 600–800 lb. of sodium cyanide per acre to which 900–1,200 lb. of ammonium sulphate is immediately added. Watson (19) has also shown the importance of thorough mixing of the fertilizer and the soil and, under certain conditions, of not applying cyanamide as a solution, but of immediately irrigating the land. He also finds that in soils containing a considerable proportion of colloids, three or four times as much cyanamide is necessary to control plant nematodes.

The practical possibilities of controlling bursate nematode larvae by means of artificial fertilizers in manure heaps, based partially on data reported in this paper, have already been discussed (8, 9, 13), as has the comparative inefficiency of these fertilizers against larvae on pastures (10).

It is interesting to note that the three nitrogenous fertilizers that lose most ammonia when mixed with feces are the most lethal, while pure ammonia water has been shown to be lethal (12).

The technique described in Part II of this series of papers (11), designed to allow the comparison of the lethal properties of various chemicals, has been used for all the substances reported upon here. The results immediately above, at, and below the lethal point are plotted by means of block diagrams on a logarithmic scale similar to those used in Part III (12). To confirm these results, cultures have been made with greater and smaller amounts than those illustrated, but as in every case they were in agreement with the diagrams they have not been recorded.

The conventions described in the third paragraph of Part III of this series (12) have been followed in this article also.

Because of the limited number of cultures which have been made and the numerous variable factors involved, it is impossible to state absolutely accurately at what proportion of fertilizer to fresh feces complete sterilization against the free-living Sclerostomes takes place. However, a study of the diagrams should indicate the proportion of fertilizer which may be expected to prove effective in practice, even against Sclerostome eggs on the edge of a midden, where conditions for development may be as favorable as in the cultures. It will be noticed that each fertilizer was re-tested in duplicate, not only at the quantities at which sterilization occurred in the preliminary cultures, but with smaller and greater quantities, to make certain that the results were not accidental. However, because of the numerous variables involved, the differences in the scale of quantities of fertilizer used have been left wide. Furthermore, it is hoped that when a study is made of the results obtained, both at the lethal and sub-lethal points, indications will be given on how various chemicals or groups of chemicals act on the eggs or larvae, and on the facility with which the larvae may avoid their action when buried in feces. In an earlier paper (11) it has been explained that occasionally a few larvae have been counted in a culture that should really have been negative,

either because some eggs were squeezed on to the side of the container when the culture was being made and so escaped contact with the chemical, or by contamination of glassware, which could only be washed and not sterilized. Sometimes this explanation does not appear to be valid, because a few larvae occur more or less regularly in cultures treated with quantities of a chemical above the apparent lethal point. In these cases it may be an indication that that chemical is very local in its action—a most important point in practice —and is not capable of reaching eggs or larvae that remain inside a lump of feces. Many of the gas-producing chemicals have this peculiarity. This difficulty should be largely overcome by using chemicals that are soluble and highly lethal in weak solutions, to allow thorough wetting of all feces. Alternatively, the survival of a few larvae may be due to the extraordinary resistance of the eggs or larvae of one or more of the numerous species which occur in horses.

When only a few larvae in a culture survive, it seems to be a point of legitimate speculation whether, if they had survived under similar conditions in nature, they would be able to survive the hazards of life in the host to maturity. While it has been absolutely impossible to attempt any infection experiments with these larvae, it has been possible to keep some of these cultures for a few months in the laboratory; in some cases, their life does not appear to have been as long as that of the controls.

Urea

Fig. 1 shows the effects of urea on the numbers and condition of the infective Sclerostome larvae obtained from 40 gm. of horse feces treated with the fertilizer within four hours of being passed. Urea ($CO(NH_2)_2$) theoretically contains 46.6% nitrogen (16).

Urea was tested dry in quantities ranging from $\frac{1}{8}$ of 1% to 20% of the feces, by weight. Sterilization against Sclerostomes was effective in any culture which was treated with 0.33 gm. or more; *i.e.*, more than 0.83% of dry urea is lethal. Quantities slightly less than this, however, were partially effective. In solution, urea is equally potent. With a 1 : 2 aqueous solution, effective sterilization is produced by 0.75 cc. or more, containing 0.28 gm. of urea; this is equivalent to 0.7% of urea to feces by weight. With a 1 : 4 solution, 1.5 cc. effects sterilization; this quantity contains 0.32 gm. of urea, or 0.8% of the weight of the feces. The results obtained with a 1 : 8 solution were less regular, but in practice it will probably be found that, at the equivalent rate of slightly over 2.0 cc. to 40 gm., effective sterilization will be effected; 2.0 cc. contains only 0.23 gm. of urea. Five cc. and 7.5 cc. of a 1 : 20 solution produced sterilization in two of the three cultures treated with both quantities, while with 10.0 cc. and more the results were uniformly effective; 7.5 cc. of a 1 : 20 solution contains 0.36 gm. of urea, equivalent to 0.9% of the weight of the feces. Since urea was so lethal, it was also tested as a 1 : 50 solution. At this strength 20.0 cc. were necessary and 15.0 cc. caused a considerable mortality. Twenty cubic centimetres of this solution contain about 0.39 gm. of urea or 0.99% by weight.

When 20.0 cc. of fluid, or even less, is added to a 40 gm. culture, there tends to be some drainage from the culture, and, therefore, some of the fertilizer may not act on the eggs or larvae. Accordingly, never more than 25.0 cc. of fluid was added.

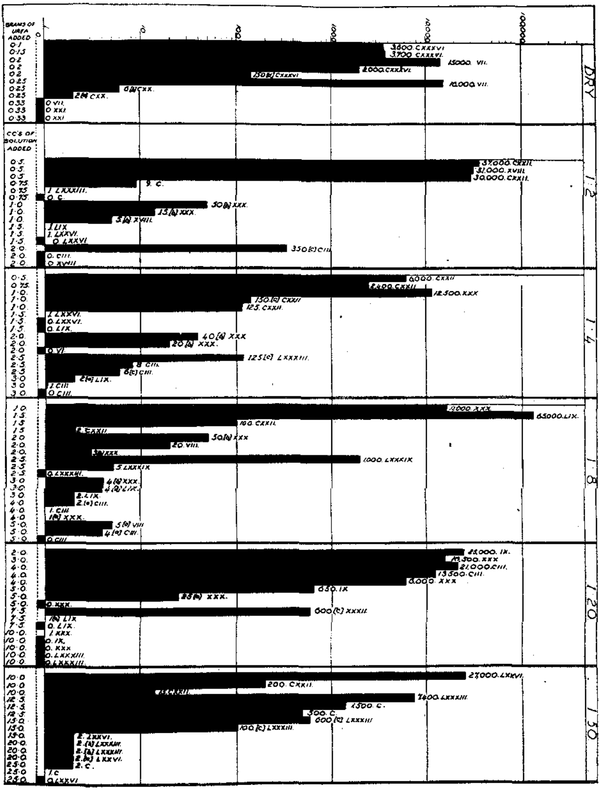

FIG. 1. *Results of tests with urea, dry and in solution. Roman numerals refer to the controls shown in Table I. a, after a number means that all, or practically all, the larvae were dead; b, that a considerable proportion were dead; c, that a few were dead. The letters have the same significance in subsequent figures.*

These figures suggest that one third of a gram of urea to 40 gm. of feces (or 0.83%) whether dry or in solution, will prove to be effective as a sterilizing agent against Sclerostomes in fresh feces.

Calurea

Fig. 2 shows the results obtained with Calurea. This fertilizer, containing 34% nitrogen, is a double salt of calcium nitrate and urea (16).

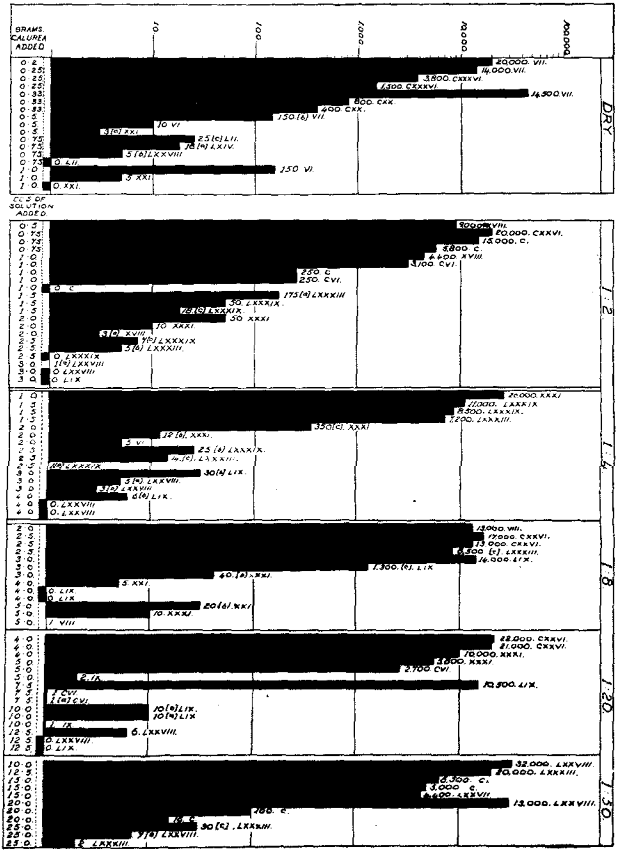

FIG. 2. *Results of tests with Calurea, dry and in solution.*

Calurea, dry, was tested in proportions of ¼ of 1% to 20% of the weight of fresh feces. The results are somewhat irregular, but suggest that 0.5 gm. in 40 gm. (or 1.25%) of Calurea would be effective. With solutions the results are also rather irregular; 1.5 cc. of a 1 : 2 solution, containing 0.57 gm., and 2.0 cc. of a 1 : 4 solution, containing 0.44 gm., approximate to the minimum quantities necessary to effect sterilization. This is equivalent to 1.33% and 1.1% of Calurea to feces. With a solution of one part in eight of water, 4.0 cc. produced sterilization; this quantity contains 0.47 gm. or 1.17% of Calurea to feces. Ten cc. or slightly less, of a 1 : 20 solution and 25.0 cc. of a 1 : 50 solution were effective; both these quantities contain 0.49 gm., equivalent to 1.23% of Calurea to feces. The results obtained with these cultures suggest that 0.5 gm. to 40 gm. of feces or 1.25% of Calurea would be effective in fresh feces.

Calcium Cyanamide

Fig. 3 illustrates the results obtained with dry powdered and granular cyanamide. Fig. 4 illustrates the results with powdered cyanamide in "solution". Calcium cyanamide ($CaCN_2$) is marketed in two forms—"pul-

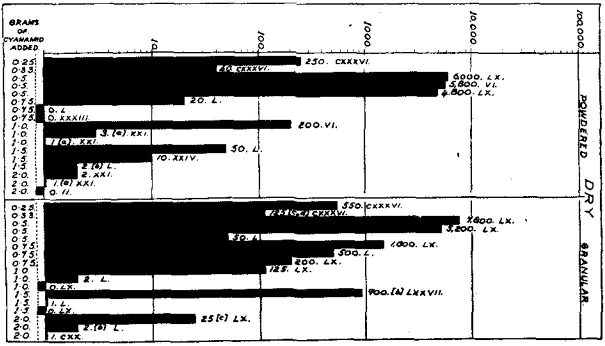

FIG. 3. *Results of tests with dry powdered, and granular cyanamide.*

verized", "powdered" or "oiled", and "granular"; the latter form is made by the use of nitrate of lime as a binding material, and obviates the difficulties of handling, which have been a disadvantage of the pulverized form as an artificial fertilizer. Since this substance may be toxic to plants, especially seedlings, during the early break-down processes, freshly treated manure should not be applied to land immediately before seeding. The usual reaction in soil is for this fertilizer to be transformed to urea and then rather slowly to nitrate. Moisture hastens the process. The sample of powdered cyanamide

which was used in these cultures contained 22% nitrogen and 70% calcium hydroxide; the granular form contained 21% nitrogen. (The results obtained with various compounds of calcium, which are comparatively ineffective, will be included in the paper on non-nitrogenous fertilizers.) Granular cyanamide was not tested in solution, since if a "solution" is required it is obviously

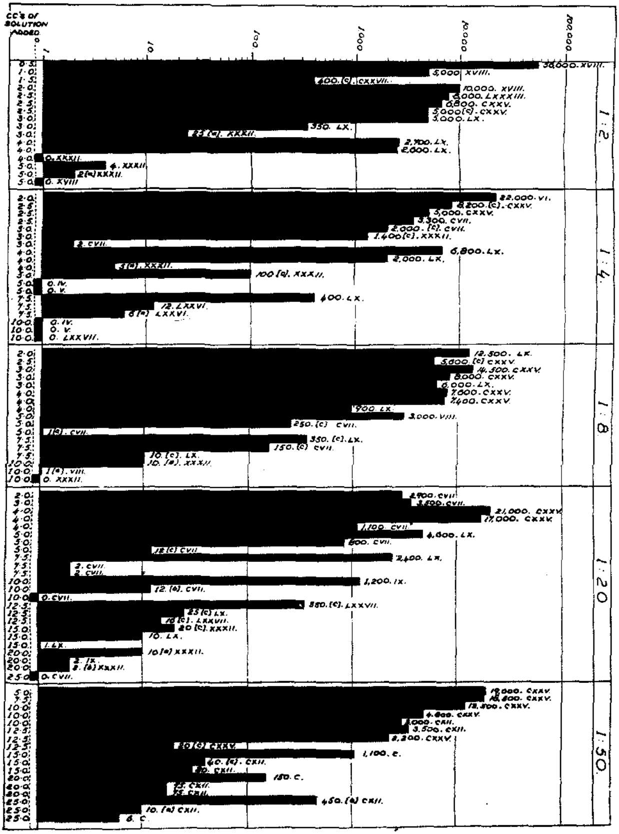

FIG. 4. *Results of tests with powdered cyanamide in "solution".*

unnecessary to use the granular form. However, 1 : 2 and 1 : 4 "solutions" of powdered cyanamide were tested, in spite of the fact that they have the consistency of mud, and would be quite useless in practice owing to the impossibility of thoroughly mixing with feces.

The results obtained with granular cyanamide were rather more irregular than those produced by the powdered form, but indicate that 0.75 gm. of dry powdered cyanamide and 1.0 gm. of the granular form are approximately the quantities necessary to sterilize a 40-gm. culture, equivalent to 1.9% and 2.5% of fertilizer to feces.

Probably owing to the difficulty of efficient mixing, 5.0 cc. of a 1 : 2 solution and the same quantity of a 1 : 4 solution had to be mixed with 40 gm. of fresh feces to kill most of the Sclerostomes; 5.0 cc. of these two solutions contain 1.9 and 1.1 gm., equivalent to 4.75% and 2.75%. Ten cc. of a 1 : 8 cyanamide-water solution was effective, while 7.5 cc. or even 5.0 cc. caused a high mortality; 10.0 cc. contain 1.2 gm., equivalent to 3.0%. The interpretation of the results obtained with 1 : 20 and 1 : 50 solutions is also difficult. It can be inferred, however, that smaller amounts of cyanamide are required with these weaker "solutions." It appears that 15 cc. is the quantity of 1 : 20 solution required to cause almost complete sterilization with regularity. This quantity contains 0.75 gm. At least 25 cc. of 1 : 50 solution is necessary for sterilization; this quantity contains slightly less than half of one gram of cyanamide, equivalent to only 1.25%.

Potassium Nitrate

Potassium nitrate (KNO_3), or saltpetre, in the pure form contains less than 14% of nitrogen. Other potash salts will be discussed in the paper dealing with non-nitrogenous artificial fertilizers. Fig. 5 shows the results obtained with potassium nitrate. Dry, this fertilizer was irregular in its action, requiring about 3.0 gm. to effect sterilization, although 2.0 gm. almost sterilized the cultures. In solution, 5.0 cc. of a 1 : 2 solution, containing 2.0 gm., is the minimum quantity at which sterilization can be expected, although smaller amounts were almost effective in a few cases, and with 7.5 cc. some larvae survived. A 1 : 4 solution was almost effective with 7.5 cc.; this contains 1.75 gm., equivalent to 4.4% of fertilizer to feces. The results with a 1 : 8 solution also were irregular, the three cultures treated with 10.0 cc. being virtually sterilized, but some larvae survived in the cultures treated with 12.5 cc.; 15 cc. of a 1 : 8 solution contains about 1.8 gm., or 4.5%. Sterilization did not occur in the cultures treated with a 1 : 20 solution. These results suggest that about 5% by weight of potassium nitrate must be added to fresh feces to kill the free-living Sclerostomes.

Sodium Nitrate

Sodium nitrate ($NaNO_3$), or Chili saltpetre, contains 15% to 16% of nitrogen, as nitrate, and therefore, is a very quick-acting fertilizer.

In addition to the cultures tabulated in Fig. 6 which show the results obtained with sodium nitrate, some cultures in which straw was incorporated,

were made with this fertilizer, and with ammonium sulphate. In both cases, the addition of straw considerably increased the proportion of fertilizer necessary.

Dry, 2.0 gm. of sodium nitrate, or 5% of the weight of feces is probably approximately the necessary proportion to obtain sterilization of fresh feces (although numerous larvae survived in one culture treated with this quantity). The quantities of 1 : 2, 1 : 4 and 1 : 8 solutions necessary to produce sterilization approximate to 7.5 cc., 10.0 cc. and 12.5 cc. containing 3.1 gm., 2.3 gm.

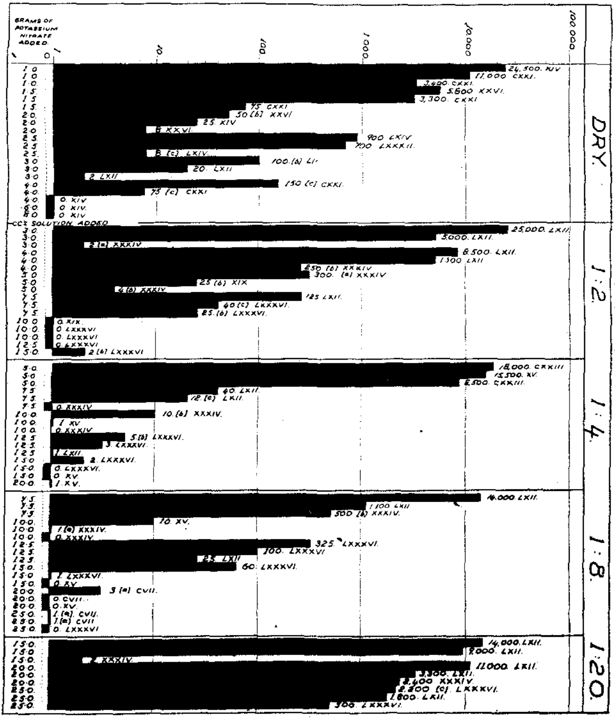

Fig. 5. *Results of tests with potassium nitrate, dry and in solution.*

and 1.5 gm. equivalent to 7.75%, 5.75% and 3.75%. However, in each case a few cultures were practically sterilized with lesser amounts. The larger quantities of a 1 : 20 solution greatly reduced the number of surviving larvae and almost effected sterilization. These cultures indicate that dry, or as a medium-strength solution, sodium nitrate is effective when used at the rate of about 5% by weight of the fresh feces with which it is mixed.

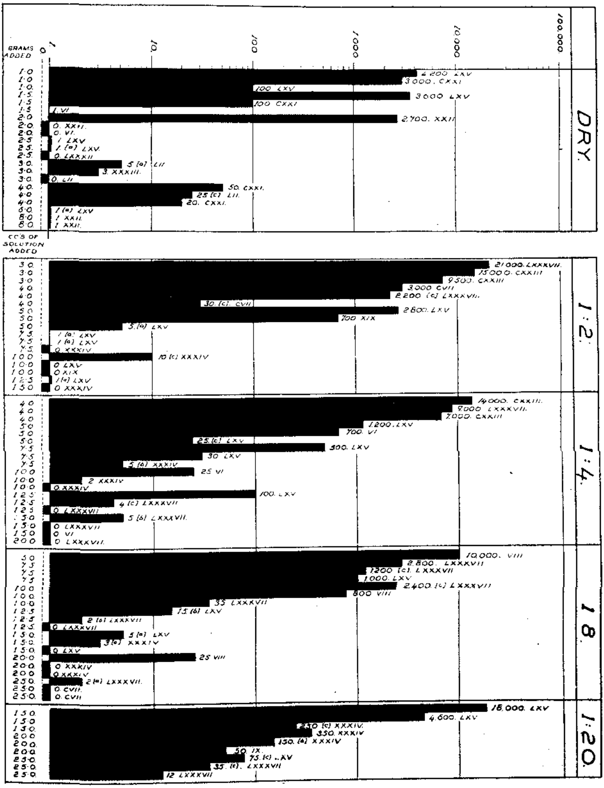

FIG. 6. *Results of tests with sodium nitrate, dry and in solution.*

Calnitro

Fig. 7 shows the results obtained with Calnitro, a fertilizer made by treating ammonium nitrate with calcium carbonate. It contains about 20% of nitrogen.

Mixed dry with feces, Calnitro produced somewhat variable results, but when the two cultures which were sterilized by 1.5 gm. are balanced against the one which was not sterilized by 2.0 gm., the assumption may be made that the Sclerostomes are killed when about 5% of fertilizer is mixed with the fresh feces. As a 1 : 2 solution, Calnitro was effective when a quantity of 7.5 cc. was used; this contains 3.0 gm. or 7.5%. As a 1 : 4 solution,

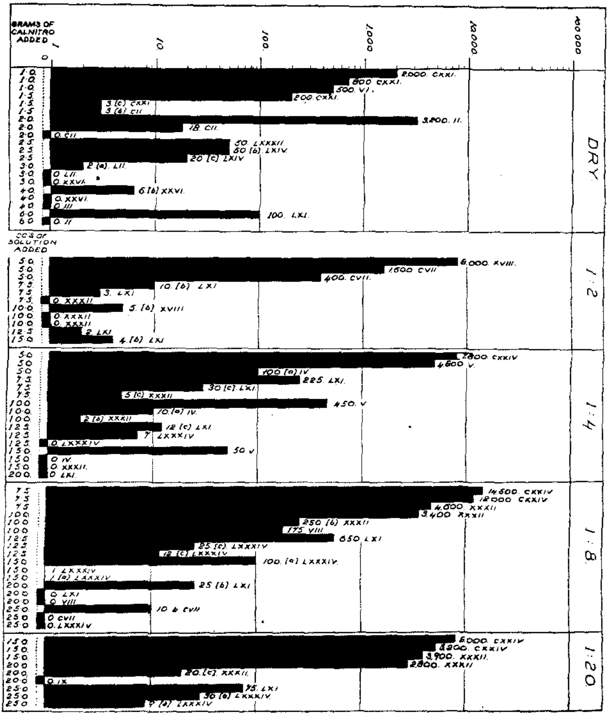

FIG. 7. *Results of tests with Calnitro, dry and in solution.*

Calnitro was more effective, but the exact value of its sterilizing effect was more difficult to interpret. Probably it approximates to 10.0 cc. containing 2.25 gm., equal to 5.6% of Calnitro to feces. Fifteen cubic centimetres of a 1 : 8 solution was effective, although less caused a considerable mortality. This quantity of solution contains 1.8 gm., or 4.5%. The quantity of a 1 : 20 solution required to sterilize 40 gm. of fresh feces is probably slightly less than 25.0 cc.; this quantity contains 1.25 gm. or the equivalent of only 3.1%.

Calcium Nitrate

Fig. 8 illustrates the sterilizing properties of calcium nitrate ($Ca(NO_3)_2$) against Sclerostome eggs or larvae when mixed with fresh feces. This fertilizer contains about 16% nitrogen.

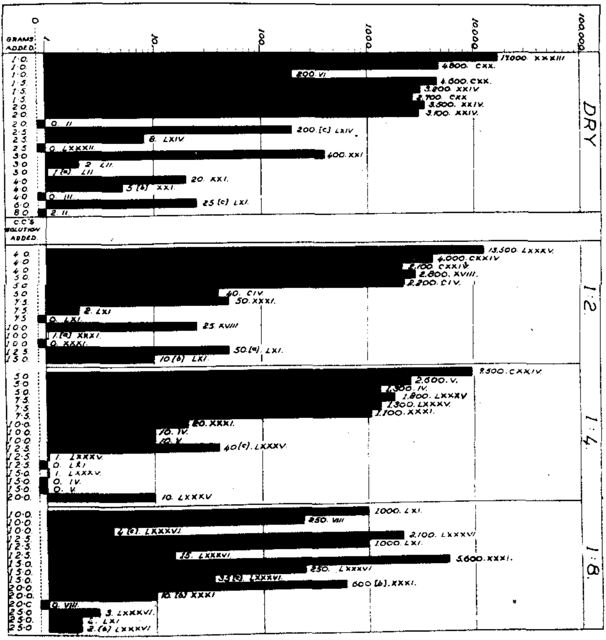

FIG. 8. *Results of tests with calcium nitrate, dry and in solution.*

Used dry, calcium nitrate is more or less effective at the rate of 2.5 gm. to 40 gm. of feces, or 6.25%. As a 1 : 2 solution, 7.5 cc. is required to obtain sterilization; this quantity contains 3.0 gm. of fertilizer or 7.5% of fertilizer to feces. Ten cubic centimetres of a 1 : 4 solution, containing 2.25 gm. is effective; this is equivalent to 5.66% of calcium nitrate to feces. As a 1 : 8 solution, the results are more irregular, 10.0 cc. sterilizing one culture, but 20.0 cc. or even 25.0 cc. being necessary for reliable results. Twenty cubic centimetres contain 2.4 gm., which is 6%. Twenty-five cubic centimetres of a 1 : 20 solution considerably reduced the number of larvae but did not produce effective sterilization. Dry, or as a medium-strength solution, calcium nitrate must, therefore, be used at the rate of about 6% of the weight of feces to kill Sclerostomes, under conditions which are probably not only ideal for the development of the larvae, but for the fertilizer to exert its lethal action.

Diammonium Phosphate

Diammonium phosphate ($(NH_4)_2HPO_4$) contains nearly 20% nitrogen and 53% phosphoric acid. Fig. 9 shows the results obtained with this fertilizer. Other phosphatic fertilizers will be considered in the paper dealing with non-nitrogenous fertilizers.

Dry, 2.5 gm. of diammonium phosphate effectively sterilized 40 gm. of fresh feces, while a few cultures were sterilized with a smaller quantity. As a 1 : 2 solution, the results were most irregular. Two cultures were completely sterilized by 7.5 cc. or 3.0 gm., but in the third culture many larvae survived; 10.0 cc. was effective. The 1 : 4 and 1 : 8 solutions also produced highly irregular results. In many cultures larvae reached the infective stage and died shortly afterwards.

Allowing for the difficulty in interpreting these results, it is probable that 10.0 cc. of a 1 : 4 solution, containing 2.2 gm. or 15.0 cc. of a 1 : 8 solution containing 1.8 gm., equivalent to 5.5% and 4.5% of fertilizer to feces, are the amounts required to obtain sterilization. With a 1 : 20 solution, only reduction of numbers and not complete destruction of the larvae was effected.

These results suggest that about 6% of diammonium phosphate to fresh feces is necessary to sterilize the latter against free-living Sclerostomes.

Nitro-chalk

Nitro-chalk also is a product made with ammonium nitrate, with finely divided chalk. Half the nitrogen, which forms 15.5% of the fertilizer, is present as ammonia and half as a nitrate. In Fig. 10 the sterilizing action of this fertilizer against free-living Sclerostomes in fresh feces is shown.

The action of dry Nitro-chalk is somewhat irregular, but the ratio required to effect sterilization appears to be about 3 : 40. As a 1 : 2 solution, the quantity necessary appears to be between 7.5 cc. and 10.0 cc.; the latter

quantity contains 4.0 gm. A 1 : 4 solution is proportionately more effective, 12.5 cc. producing sterilization and 7.5 cc. and 10.0 cc. heavy death rates; 12.5 cc. contains 2.8 gm., or 7%. Fifteen cubic centimetres of a 1 : 8 solution, which contains only 1.8 gm., was sufficient to produce effective results; this figure is equivalent to 4.5%. The largest quantities used of a 1 : 20 solution only reduced the number of Sclerostome larvae. These results

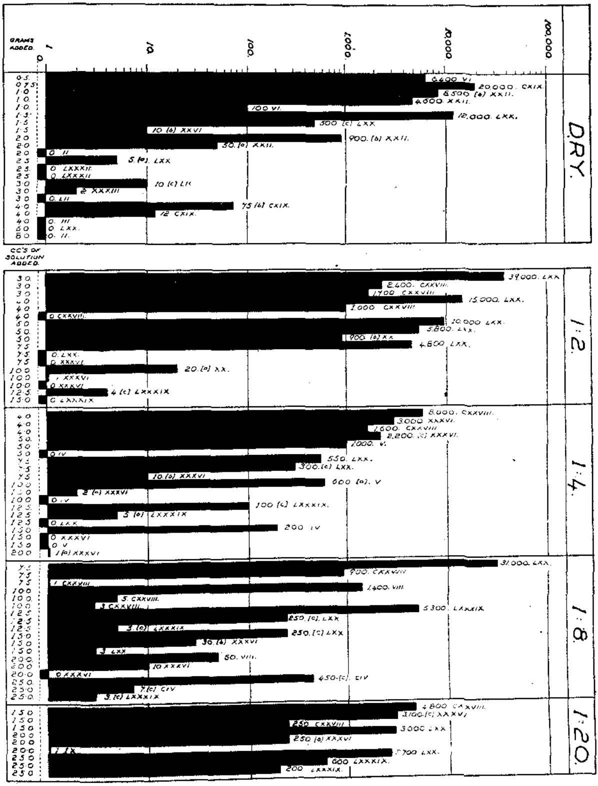

FIG. 9. *Results of tests with diammonium phosphate, dry and in solution.*

suggest that Nitro-chalk should not be used as strong or very weak solutions, and that even under optimum conditions, it is among the least lethal of the nitrogenous fertilizers.

Ammonium Sulphate

Ammonium sulphate ($(NH_4)_2SO_4$), one of the original artificial nitrogenous fertilizers, contains about 20% of nitrogen. The resnlts obtained with this fertilizer are shown in Fig. 11.

Dry ammonium sulphate at the rate of 7.5% may be expected to produce sterilization. In solution, 7.5 cc. of a 1 : 2 solution containing 3.0 gm., and 12.5 cc. of a 1 : 4 solution—equivalent to 2.8 gm.—appear to be the minimum quantities at which sterilization regularly occurs (equivalent to

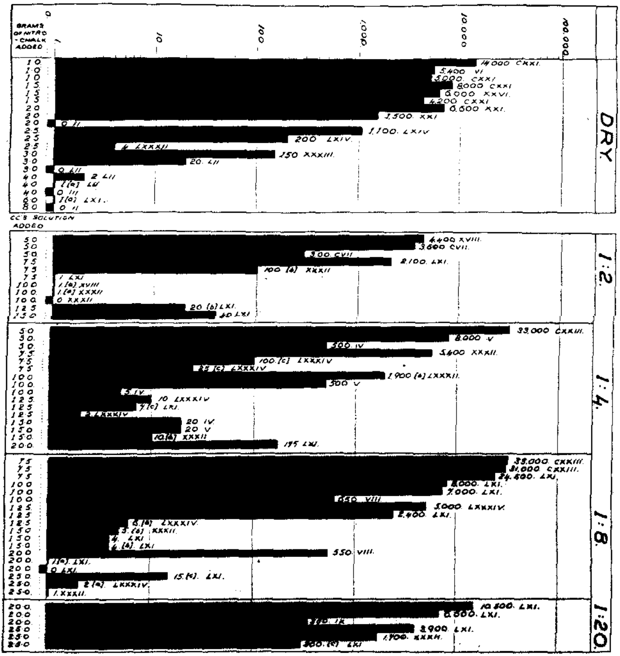

FIG. 10. *Results of tests with Nitro-chalk, dry and in solution.*

7.5% and 7%) although some cultures were sterilized with smaller amounts. The results with a 1 : 8 solution were also most irregular, but 20.0 cc. containing 2.4 gm. (6.0%) appears to be the least amount upon which reliance may be placed. Even 25.0 cc. of a 1 : 20 solution failed to reduce Sclerostome larvae to numbers small enough to have no importance in practice.

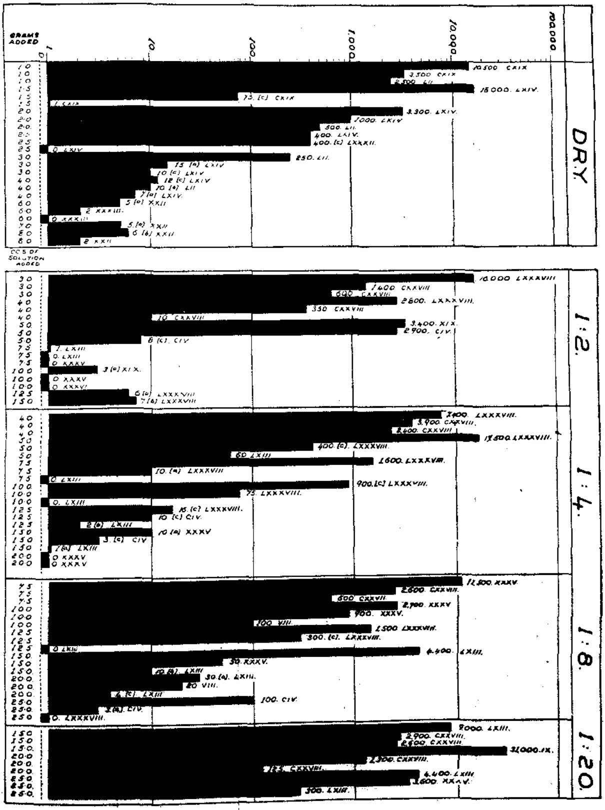

FIG. 11. *Results of tests with ammonium sulphate, dry and in solution.*

TABLE I

KEY TO FIGS 1–10

Series number	Date cultures made	Days kept in C.T. room	Average number of larvae isolated
II	August 27, 1934	10	23,000
III	August 27	17	25,000
IV	September 19	11	1,100
V	October 5	10	7,600
VI	December 12	19	21,000
VII	January 4, 1935	11	21,000
VIII	January 4	24	21,000
IX	February 13	31	44,000
XIV	May 21	23	29,000
XV	May 21	36	18,000
XVIII	May 31	32	60,000
XIX	May 31	32	13,000
XX	May 31	33	12,000
XXI	June 5	39	24,000
XXII	June 5	39	25,000
XXIV	July 9	18	72,000
XXVI	July 9	18	48,000
XXX	July 12	21	27,500
XXXI	July 12	77	35,000
XXXII	July 15	77	15,000
XXXIII	July 19	14	24,000
XXXIV	July 22	82	32,000
XXXV	July 22	84	23,000
XXXVI	July 23	92	23,000
L	November 21	28	25,000
LII	November 22	25	62,000
LIX	December 17	28	32,000
LX	December 23	17	45,000
LXI	December 27	21	42,000
LXII	December 27	27	16,000
LXIII	January 2, 1936	27	27,500
LXIV	January 2	29	36,000
LXV	January 2	32	11,500
LXX	January 16	19	64,000
LXXVI	January 30	20	5,000
LXXVII	January 30	22	33,000
LXXVIII	January 30	31	30,000
LXXXII	February 18	22	16,500
LXXXIII	March 3	13	25,000
LXXXIV	March 3	15	12,500
LXXXV	March 4	14	31,000
LXXXVI	March 4	19	33,000
LXXXVII	March 4	20	38,000
LXXXVIII	March 5	22	15,000
LXXXIX	March 5	25	26,500
C	March 26	21	3,600
CII	April 1	20	27,500
CIII	April 1	22	26,000
CIV	April 7	23	21,500
CVI	April 16	15	13,000
CVII	April 16	19	17,000
CXII	April 24	24	7,800
CXIX	May 15	27	59,000
CXX	May 15	29	24,000
CXXI	May 15	32	42,000
CXXII	May 22	17	4,400
CXXIII	May 26	21	49,000
CXXIV	May 26	23	40,000
CXXV	May 26	25	18,500
CXXVI	May 26	28	26,500
CXXVII	June 9	16	11,000
CXXVIII	June 9	18	10,000
CXXXVI	July 2	12	2,200

Conclusion

The proportion of nitrogenous fertilizers to feces shown in this paper as necessary to effect sterilization would, in general farming practice, be too high to be used, if all the feces had to be treated, but in a well-built manure heap, only that manure which was put on the bottom, top or sides would have to be treated, because Sclerostome larvae are unable to survive the heat of fermentation, and probably the lack of oxygen and harmful products of decomposition, in the centre of a manure heap.

Acknowledgments

It is a pleasure not only to be able to record continued help from the firms mentioned in Part II of these papers (11), but to be able to thank Canada Packers, Limited, The Chemical and Metallurgical Corporation Limited, Tobacco By-products and Chemical Corporation Inc. (U.S.A.) and British Alkaloids Limited (England) most sincerely for their generous help.

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Canadian Journal of Research

Issued by The National Research Council of Canada

VOL. 15, SEC. D. AUGUST, 1937 NUMBER 8

A PRELIMINARY ACCOUNT OF THE UNIONIDAE OF ONTARIO[1]

By A. La Rocque[2] and J. Oughton[3]

Abstract

This study is based on an examination of museum specimens and records in the literature. The Unionidae of Ontario comprise 45 species and 18 varieties. Their distribution in the various drainages is shown in a table and the fauna of each area is discussed. The doubtful species and three erroneous records are noted.

Introduction

As an introduction to studies of the distribution of the Unionidae of Ontario, lists that have been published for the Toronto, Hamilton and Ottawa areas and data from more comprehensive works have been brought together, and the specimens on which the lists were based have been examined wherever possible. The taxonomy has been brought up to date (under the direction of Dr. Henry Vander Schalie) and an attempt has been made to indicate briefly the known distribution. Although a large proportion of the shells collected in Ontario have been examined, some species still remain in the "doubtful" list.

The freshwater mussels and clams of Ontario can be separated into two families, the Unionidae (pearly mussels) and Sphaeriidae (fingernail clams). They may be briefly described thus:

Family Unionidae. Length, 1 to 8 in. Shape, varied; some species almost circular, others oval, pendant-like or elongate; some have grooves or knobs on the surface. Color, externally usually brown, green, straw or yellow; sometimes with clearly marked radiating lines; internally white, bluish, silver or rose; often with iridescent hues.

Family Sphaeriidae. Length less than 1 in. Shape, oval or oblong, chiefly, with no conspicuous ornaments. Color, externally straw or light brown; internally white or bluish white.

This account deals with the Unionidae only.

[1] *Original manuscript received April 24, 1937.*
Contribution from the Royal Ontario Museum of Zoology, Toronto, and the National Museum of Canada, Ottawa, Canada.

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Sources of Material

The specimens which have been examined are in the following museums:

1. Uniyersity Museums (including the Bryant Walker Collection, University of Michigan).
2. The National Museum of Canada.
3. Royal Ontario Museum of Zoology.

These, together with the literature listed below, form the actual basis for this list.

The collection at Ottawa has been partly determined by Simpson and partly by Bryant Walker. Representatives of species in the Toronto collection have been examined by Dr. Vander Schalie.

These collections contain moderately extensive series of shells from the settled parts of Ontario but only a sparse representation from that vast area of the province that is west and north of the Georgian Bay drainage.

We are unable to divide certain genera represented in Ontario into well-marked species. Such "difficult" groups are *Anodonta* and *Lampsilis*. We have not been able to consider their distribution in detail. Likewise *Obovaria* and *Pleurobema* are "difficult" but of less importance since they are not common mussels throughout the province.

The size and shape of the shell in the Unionidae have been shown by Ortmann, Grier and others to be correlated with environmental differences. For example, the shell of a mussel living in a large lake may be dwarfed when compared with that of the same species dwelling in a large slow river. Specimens collected by us in the Niagara River about a mile or two upstream from the Falls, where the current is moderately fast, show similar dwarfing to that induced by lake conditions. We have listed several but not all of these "environmental varieties".

It should be noted that *Amblema costata* (Raf.) and *Amblema plicata* (Say) are herein noted separately, although it seems to us that they are not specifically distinguishable.

We concur in the opinion of Latchford who was the first to point out that *Lampsilis borealis* (A. F. Gray) (7) is no more than a variety of *Lampsilis radiata*, and have treated it accordingly herein. It is interesting to note that the original figures of this "species" were drawn by Justice Latchford and that the initials A. G. on the plate refer to Mr. A. Grignard, the lithographer, and not to A. F. Gray who drew up the description.

A Comparison of Distribution in the Various Ontario Drainages

For this purpose, we have included only 40 species and 14 varieties, having eliminated certain closely related species. The species and varieties of *Anodonta* have been considered as but one species since we feel that many of the species recorded are not valid.

I. *Hudson and James Bay Drainage*

The Hudson and James Bay drainage has a moderately large Unionid fauna but most of the species have been recorded only for Manitoba. This fauna seems to extend only slightly into Ontario. *Lasmigona complanata* may be taken as an example. In Ontario this species is definitely known only from the extreme western portion -Lake of the Woods (Dawson (4)), and Winnipeg River (Latchford (11), Mozley (13)). In Ontario waters of the Hudson and James Bay drainage in general there is apparently a very small mussel fauna, namely eight species and two varieties.

II. *Lake Superior and Drainage*

There are records for only eight species and two varieties—a list equal to that of the above drainage.

III. *Lake Huron and Drainage*

There are 24 species and eight varieties definitely known from this section. It is curious to note that, while *Elliptio complanatus* (Dillw.) is generally common in the Lake Huron drainage, it is apparently very rare in one part, Lake Simcoe. Dr. E. M. Walker, who has for a long time been acquainted with this area, has never found it in the lake. But Rawson (17) has found specimens in the mouths of creeks draining into Lake Simcoe.

IV. *Lake St. Clair, Detroit River, Lake Erie and their Drainages*

These, the most southern waters of the province, contain the greatest variety of mussel fauna, having 34, 26 and 34 species, respectively. Seven species on our list are entirely confined to these drainage areas. *Elliptio complanatus* (Dillwyn) is either absent or very rare here. Ortmann in his Monograph (15, p. 110) says concerning this species: "It has never been found in Lake Erie . . . ."

V. *Niagara River and Tributaries*

The fauna comprises 26 species and four varieties.

VI. *Lake Ontario and Drainage*

We have records for 17 species and four varieties. Two of these species, namely *Leptodea fragilis* and *Proptera alata* are very rare in Lake Ontario. They have been found only in the western end of the lake. It is possible that these two species have migrated there by way of the Welland Canal.

VII. *St. Lawrence (in Ontario) and Ottawa Rivers and Tributaries*

In this drainage section, there are 18 species and five varieties known. Part of this fauna shows affinities with that of the Atlantic slope drainage. For example, *Alasmidonta undulata* is confined, in Ontario, to this drainage section. It is found in the Ottawa River even into the headwater creeks in Algonquin Park but has not been reported from adjacent drainages.

The genus *Lampsilis* exhibits considerable variation in this section. The most "typical" specimens of *L. radiata* have been collected in the Ottawa River, although it has been found in other waters of Ontario.

Summary

1. The pearly freshwater mussel fauna of Ontario comprises 45 species and 18 varieties and of these the greatest number are to be found in the southernmost waters.

2. Seven species are confined in Ontario to Lake Erie, Lake St. Clair, Detroit River, and their drainages.

3. Our present knowledge of the distribution of these mussels, as shown briefly by the list, indicates that the fauna is probably composed of different elements having different affinities and origins. During post-glacial time, when Ontario waters were connected with the Mississippi drainage and with the Atlantic drainage, elements of the Ontario naiad fauna probably migrated from the south and from the east.

Doubtful Species

Anodonta imbecillis Say

This species is recorded from the Michigan side of Lake Erie but as yet we have seen no authentic specimens from Ontario.

Anodonta pepiniana Lea

The status of this species is still in doubt. It may be only a stunted form of *Anodonta grandis*. It has been reported from Lake Superior, from "upper and middle St. Lawrence drainage."

Dysnomia sulcata delicata (Simpson)

This variety is known to occur in the Detroit River but as yet there are no definite records from the Ontario side.

Fusconaia subrotunda (Lea)

The species has been recorded for the Detroit River but so far we have seen no specimens from Ontario. One specimen (NMC 2417) from the Grand River, originally recorded as this species, is *Pleurobema cordatum coccineum*.

Fusconaia undata (Barnes)

This species also has been recorded for the Detroit River but so far we have seen no authentic specimens from Ontario. Two specimens from Port Dover, Lake Erie (NMC 2457) were identified as this species by Simpson, but Walker, who examined them later, decided that one of them was "*Quadrula flava*" and labelled the other "may be *Quadrula undata* Bar. but very doubtful, rather *flava*". Dr. Henry Vander Schalie, to whom this lot was submitted, assures us that both specimens should be referred to *flava parvula*.

Lampsilis ochracea (Say)

All Ontario records appear doubtful.

Leptodea leptodon Raf.

Is recorded for Detroit River. We have seen no authentic Ontario specimens.

Quadrula pustulosa schoolcraftensis (Lea)

Recorded (as *pustulosa prasina*) for Lake Erie by Goodrich and Vander Schalie. We have seen no authentic Ontario specimens.

Simpsoniconcha ambigua (Say)

The species is known to occur in Lake Erie and the Detroit River but as yet there are no definite records from the Ontario side.

FAMILY MARGARITANIDAE:

Margaritana margaritifera (L.)

Recorded for the St. Lawrence River in Quebec but not known for Ontario.

Erroneous Records

The following species have been recorded for Ontario in error:

Actinonaias ellipsiformis (Conrad)

Simpson lists this species for "Southern Canada" and it is recorded by Robertson for the Toronto region, the record being based on specimens in the Brodie collection. This collection is now in the Royal Ontario Museum of Zoology and contains no specimens of this species; neither are there any specimens in the National Museum. Moreover, Dr. Vander Schalie is convinced that this is an error and suspects that specimens of *Micromya iris* were mistaken for this species, which is readily done by one not familiar with the group.

Elliptio crassidens (Lamarck)

Nicholson (1872) lists this species for Toronto Bay, and Robertson (Nat. Hist. Toronto Region, 1913, p. 292) repeats the Nicholson record, but with a question mark. We have never seen specimens of *E. crassidens* from Toronto Bay or from any other part of Ontario. It seems probable, therefore, that the original record was based upon a misidentification.

Pleurobema clava (Lamarck)

The basis for this record is the citation of Call (Trans. Acad. Sci. St. Louis, Vol. 7, p. 9, 1895) who says in part: ". . . . it ranges, as specimens at hand prove, from western New York to Ottawa river, Canada, thence west to Illinois and south to Middle Alabama . . . . " We believe the Ottawa River record to be erroneous, since the species has never been found there to our knowledge although local collectors have been fairly active both before and since the publication of Call's record. So far we have seen no authentic specimens from Ontario.

TABLE I

DISTRIBUTION OF SPECIES

Species	Hudson Bay Drainage	Lake Superior	Lake Superior Drainage	Lake Huron	Lake Huron Drainage	Lake St. Clair	Lake St. Clair Drainage	Detroit River	Lake Erie	Lake Erie Drainage	Niagara River and Trib.	Lake Ontario	Lake Ontario Drainage	St. Lawrence and Trib.	Ottawa Riv. and Trib.
Actinonaias carinata (Barnes)						M	*		M	*	*	*			
Alasmidonta calceolus (Lea)				*		*	*	*	*	*	*	*			*
Alasmidonta marginata (Say)				*	*	*	*	*	*	*	*	9		*	*
Alasmidonta undulata (Say)														*	*
Amblema costata (Raf.)					*	*	*	*	*	*	*				A
Amblema plicata (Say)				*		*		M	*		*				A
Anodonta cataracta Say															
Anodonta grandis Say															
Anodonta grandis benedictensis Lea															
Anodonta grandis footiana (Lea)	The species have been recorded for Ontario. The genus is generally distributed in all drainages.														
Anodonta grandis gigantea (Lea)															
Anodonta implicata Say															
Anodonta kennicottii Lea															
Anodonta marginata Say															
Anodontoides ferussacianus (Lea)				*	*		*		*	*		*	15	*	*
Anodontoides ferussacianus buchanensis (Lea)				*		*			*						
Anodontoides ferussacianus subcylindraceus (Lea)			*	*	*	*	*	*	*	*	*	*	*	*	*
Carunculina parva (Barnes)									*	*					A
Cyclonaias tuberculata (Raf.)							*	M	*						A
Dysnomia rangiana (Lea)						*		*	*						A
Dysnomia triquetra (Raf.)				*		*	*	*	*	*					A
Elliptio complanatus (Dillwyn)	*	*	20	*	*	A?	A?		*?	*?	*	*	*	*	*
Elliptio dilatatus Raf.				*	*		*	*		*	*	*	*?	*	*
Elliptio dilatatus sterkii Grier				*		*			*		*		*?		A
Fusconaia flava (Raf.)					5?		*			*	*				A
Fusconaia flava parvula Grier				*		*			*						A
Lampsilis cariosa (Say)													*		*
Lampsilis fasciola (Raf.)				*		*	*	*	*	*	*				A
Lampsilis radiata (Gmelin)	1		*	*	*		*			*?		*		*	*
Lampsilis radiata borealis (A. F. Gray)	*?		*		*?								*?		*
Lampsilis siliquoidea (Barnes)	*	*		*	*	*	*	*	*	*	*	*		*	*
Lampsilis siliquoidea rosacea (DeKay)	*	15	15	*	*	*			*		*	*	15	*	
Lampsilis superiorensis (Marsh)	*	M	*		*										
Lampsilis ventricosa (Barnes)	*	*?		*	*	*	*	*	*	*	*		*?	*	*
Lampsilis ventricosa canadensis (Lea)	M			*		*			*			*?		*?	*?
Lasmigona complanata (Barnes)	*								*?						A
Lasmigona complanata katherinae (Lea)		*?		*											A
Lasmigona compressa (Lea)	*		M		*	*	*	*	*	*	*	*?	*	*	*
Lasmigona costata (Raf.)			15		*	*	*	*		*	*		*		*
Lasmigona costata eriganensis Grier						*			*		*				A
Leptodea fragilis (Raf.)						*	*	*	*	*	*	*			*
Leptodea fragilis lacustris F. C. Baker						*			*		*?	*			A
Ligumia nasuta (Say)				*		*		*	*	*	*	*	*		A
Ligumia recta (Lam.)				*	*	*		*	*	*	*	*		*	*
Ligumia recta latissima (Raf.)										*			*		*
Micromya fabalis (Lea)							*	*	*						A

TABLE I—*Concluded*

DISTRIBUTION OF SPECIES—*Concluded*

Species	Hudson Bay Drainage	Lake Superior	Lake Superior Drainage	Lake Huron	Lake Huron Drainage	Lake St. Clair	Lake St. Clair Drainage	Detroit River	Lake Erie	Lake Erie Drainage	Niagara River and Trib.	Lake Ontario	Lake Ontario Drainage	St. Lawrence and Trib.	Ottawa Riv. and Trib.
Micromya iris (Lea)					*	*	*		*	*	*	*	M		
Micromya iris novi-eboraci (Lea)					*	*	*	*	*	*	*			*	
Obliquaria reflexa (Raf.)							*		*	*					A
Obovaria leibii (Lea)						*	20?	*	*	*					A
Obovaria olivaria (Raf.)					*	*	*	*	*	20	*			*	*
Pleurobema cordatum coccineum					M	*	*	*	*	*	*		20?		A
Pleurobema cordatum pauperculum						*			*		19?				A
Propiera alata (Say)	*?			*		*	*	*	*	*	*	*			*
Proptera alata megaptera (Raf.)				*?			*		*?	*	*				*
Ptychobranchus fasciolare (Raf.)						*	*	*	*	*	*				A
Quadrula pustulosa (Lea)							*		*	*	*				A
Quadrula quadrula (Raf.)							*		*	*	*				A
Strophitus rugosus (Swain.)				*	*	*?	*	M	*	*	*	*?	M	*	*
Truncilla donaciformis (Lea)						*			*	*	*?				A
Truncilla truncata (Raf.)						*	*		*	*					A
TOTALS															
Species	8	4	5	16	16	23	27	23	30	29	24	13	8	12	17
Species (? Record doubtful)	1	1	0	0	1	1	1	0	2	2	1	2	3	0	0
Varieties	2	1	3	7	4	11	4	2	10	4	6	3	3	3	4
Varieties (? Record doubtful)	1	1	0	1	1	0	0	0	1	0	2	1	2	1	1

**Specimens in National Museum of Canada or Royal Ontario Museum of Zoology.*
M—*In University Museums, University of Michigan.*
1, etc.—*Record in literature listed.*
?—*Doubtful record.*
A—*Absent.*

Acknowledgments

We are much indebted to Dr. Henry Vander Schalie for assistance in matters taxonomic, and to Chief Justice Latchford for his helpful suggestions.

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SOME OBSERVATIONS ON THE OVERWINTERING OF CERTAIN HELMINTH PARASITES OF SHEEP IN CANADA[1]

By Henry J. Griffiths[2]

Abstract

The helminths, *Meniezia expansa, Ostertagia circumcincta, Nematodirus filicollis* and *Trichostrongylus colubriformis* demonstrated the ability to survive a normal Canadian winter and to cause infection of sheep when ingested during the following spring. Temperatures during the four-month period ranged from −15° F. to 52.5° F. It is concluded that pastures cannot be considered free from these helminths after exposure to such temperatures.

Introduction

While it is an accepted principle of control of helminth parasites in domestic animals that prevention of infection is greatly preferable to destruction of the helminth after it has gained access to the host, it is true that most reliance has so far been placed on anthelmintic medication. This is unfortunate for many reasons, among the most important of which are that few anthelmintics are efficient and all are more or less toxic, and very often much damage has been done by the young parasite before it becomes susceptible to the action of the drug.

This state of affairs has arisen largely because of our lack of knowledge of the bionomics of the helminths and our consequent inability to apply rational scientific methods of control. At the present time, however, with the limited knowledge available, much can be done by general sanitation, light stocking pasture rotation, bare lots, and so on, in reducing infestations.

The importance of a helminthic infestation almost always depends upon the number of worms present, and, as no worm can multiply entirely inside the body, the extent of the infection depends ultimately on the number of eggs or larvae on the pasture. It is accordingly essential to have an adequate knowledge of the conditions under which these eggs and larvae will survive in the free state. The following observations on the effects of a Canadian winter on some of these parasites, are presented as helping to throw some light on the free life of these animals.

Results were obtained incidentally, while attempting some pure-strain infestations of sheep with a single helminth. Records of the previous season's experiments on these plots, and the biological data revealed, seemed of sufficient significance to be recorded.

Experimental

Plots

The plots utilized in these trials were small, averaging about 350 sq. yd each. They were well fenced, and in the early part of the summer yielded fair pasture, comprising a mixture of the following common grasses: white

[1] *Manuscript received July 5, 1937.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College Que., with financial assistance from the National Research Council of Canada.
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Dutch clover, red top, timothy, Kentucky bluegrass, couch grass and cocksfoot. After a short period of pasturing, supplemental feeds were supplied owing to the exceedingly heavy concentration of sheep on so small an area. Experimental plots were separated from each other by a plot which was not in use. This precaution avoided danger of fecal contamination.

Stock

The lambs employed in this work were from the experimental flock of this Institute. They were born and raised under as helminth-free conditions as practicable, being permitted to suckle their mothers only at certain periods of the day, following the washing of the ewe's udder. Under these conditions lambs have been raised free of bursate nematodes although, in most cases, coccidiosis and an infestation of *Strongyloides papillosus* were not eliminated.

Lambs raised under these conditions have not shown the normal growth and weight that would be observed with lambs running with their mothers at pasture during the summer. However, the lambs have been in good health and have not shown any deficiency symptoms or other physiological disorders.

Fecal Tests and Post-mortem Examinations

Feces were taken, when necessary, directly from the rectum of the individual. The usual centrifugal methods were employed for diagnostic purposes. Post-mortem examinations were carried out in the usual routine by sedimentation of the contents of the intestine; the helminths obtained were preserved in 70% alcohol.

Observations and Results

Plot I. During the summer of 1935, this pen pastured a ewe and a yearling which were on salt lick trials. Throughout the summer fecal tests revealed the sheep to be harboring at least two species of strongyles, and on one occasion tapeworm ova were recorded. These sheep were removed from pasture early in October, 1935. On May 27, 1936, two lambs were put out to pasture in this pen. One lamb was fed a culture of *Haemonchus contortus* larvae, the other being used as a control. Previous to use on this work the lambs were tested and found positive for coccidia and *Strongyloides papillosus* only. On June 6, fecal examination showed the presence of *S. papillosus* and coccidia. On June 30, the experimentally infected lamb showed ova of two species of strongyles and coccidia, the control lamb yielding ova of two species of strongyles, of *S. papillosus* and of a capillarid, coccidia oöcysts also being present. (At this date, June 30, the feces of five control lambs were examined and found to yield only ova of *S. papillosus* and oöcysts of coccidia.) On July 22, fecal tests were made of the two experimental lambs, and analysis revealed the ova of *Nematodirus* sp., strongyles (probably *H. contortus*), and coccidia oöcysts. This test was repeated on July 23 with

identical recordings. This infestation increased and post-mortem results from these lambs were as follows:—

Lamb experimentally infected with *H. contortus:* Killed August 28, 1936	Control lamb, killed July 30, 1936
Ostertagia circumcincta	*O. circumcincta*
Nematodirus filicollis	*N. filicollis*
Haemonchus contortus	*Trichostrongylus colubriformis*
Moniezia expansa	*M. expansa*

Plot II. Throughout the summer of 1935 this plot pastured a ewe, a yearling and a lamb on salt lick experiments similar to those in Plot I. Numerous fecal examinations throughout the summer showed these individuals to be harboring a heavy infestation of helminths, tapeworm ova and four different species of nematode ova being observed. On September 30, 1935, the lamb died, post-mortem examination yielding *Trichostrongylus* sp., *Monodontus trigonocephalus*, and *Chabertia ovina*. During the early part of October, 1935, the ewe and yearling were removed from pasture to winter quarters.

On May 27, 1936, two lambs were pastured in this pen, one animal being fed a pure culture of infective larvae of *C. ovina*, the other being kept as a control. The lamb drenched with the infective larvae died during the last week in June. Unfortunately it was impossible to make an autopsy. Fecal examination of these two lambs on June 11, 1936, revealed the presence of coccidia oöcysts only. On June 30, the control lamb yielded ova of one species of strongyle and of *S. papillosus* on fecal test. (Five control lambs were tested and found to yield only *S. papillosus* ova and coccidia.)

On July 22, 1936, the remaining lamb, on fecal examination, showed heavy infestation of *Moniezia* sp., and ova of a species of strongyle. On July 28, 1936, this lamb was slaughtered, an autopsy yielding the following helminths: *Ostertagia circumcincta, Nematodirus filicollis, Trichostrongylus colubriformis, Moniezia expansa, Strongyloides papillosus.*

Plot III. This plot also was utilized for salt lick trials during the summer of 1935, pasturing a ewe, a yearling and a lamb. Numerous fecal examinations of these individuals throughout the summer showed them to be harboring three species of strongyles and tapeworms. These sheep were removed from pasture early in October, 1935.

Two lambs were pastured in this pen on May 27, 1936. Fecal tests on these animals on June 11, 1936, showed an infestation of coccidia only. On June 30, 1936, the examination revealed ova of a species of strongyle and of *S. papillosus* and coccidia oöcysts. (Five controls examined on the same date were found to be carrying *Strongyloides* ova and coccidia only.) Fecal examination of these lambs on July 22, 1936, showed them to be harboring

two species of strongyles, *S. papillosus* and coccidia. Post-mortem examination of these lambs yielded the following helminths:

Killed July 22, 1936. *O. circumcincta*, *N. filicollis*, *S. papillosus*.

Killed July 24, 1936. *O. circumcincta*, *N. filicollis*, *M. expansa*.

Controls

Of the 11 lambs available for experimental purposes, five were kept as controls throughout the summer of 1936, to be used later for other purposes. These five lambs and those selected for use on the experimental plots were raised under identical conditions. They had every opportunity of picking up an infestation of helminths should the technique employed in raising helminth-free lambs have proved unsatisfactory. All the lambs were recorded as carrying *S. papillosus* and a coccidial infection at some time or other and, consequently, these two parasites were disregarded throughout the investigation.

Until June 29, 1936, these five control lambs were kept indoors in concrete pens. On that date the lambs were put out in small paddocks which were known not to have been pastured by sheep for many years. Fecal tests were made of these control lambs at irregular intervals, and at no time were any ova observed except *S. papillosus*. Coccidia oöcysts were observed on most occasions.

Resistance to Winter Temperatures

Certain helminths mentioned in this paper have been shown to possess the ability to resist the low temperatures of a Canadian winter. These helminths not only have the power to withstand comparatively low temperatures, but also appear to possess the ability to withstand freezing and thawing to a considerable extent.

Whether this resistance mechanism is functional in the ovum or the infective larvae is still debatable, but from the observations of other investigators it is probable that infective larvae would withstand the rigors of winter to a far greater extent than would the ova.

The maximum and minimum temperatures for a four-month period of the winter of 1935–36, are charted in Fig. 1. These temperatures were obtained through the courtesy of the Department of Horticulture, Macdonald College, McGill University.

It will readily be noted from the graphs that some sharp rises and falls in temperature were recorded during the four-month period. The maximum and minimum temperatures, as indicated in the graphs, for December, 1935, and January and February, 1936, will be observed to be mostly below 32° F. At several periods during these months the mercury dropped to zero or well below, the minimum temperature recording well below zero for several days at a time in some instances. In the first few days of March the temperature dropped to −11° F. This was followed by milder weather, though at no period during the month did the minimum recorded temperature rise much above freezing point.

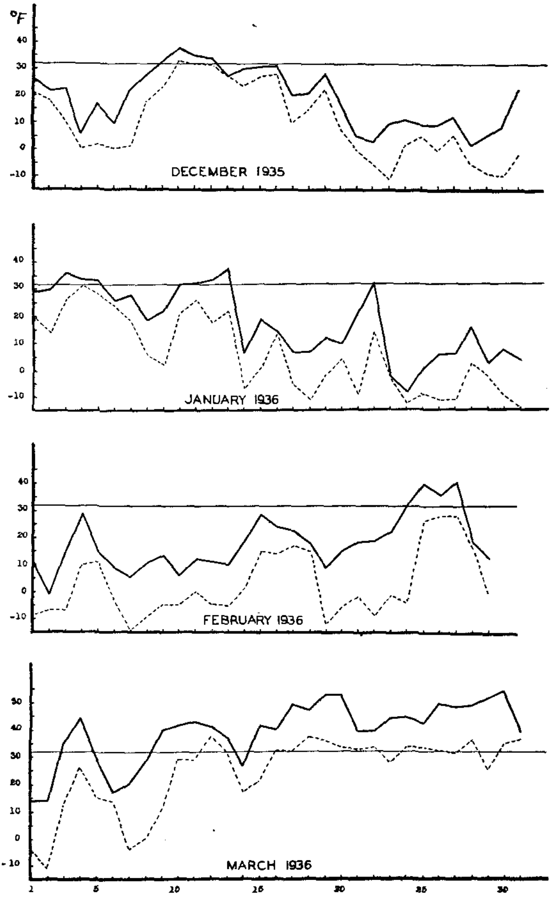

FIG. 1. *Graphs showing maximum and minimum temperatures for a four-month period in the winter of 1935–36. Maximum temperatures indicated by continuous line, minimum by dotted line.*

December, January and February were slightly colder than the average for the island of Montreal, although March was warmer (Table I). The precipitation for January and February was normal—both rain and snow—but in December both rain and snow were considerably less than usual, and in March rain was above average while snowfall was below average. It seems therefore that the winter of 1935 in this district was slightly more severe than normal and that the worms which survived this winter might be expected to survive any normal winter in the Montreal area.

TABLE I

TEMPERATURE AND PRECIPITATION

—	December		January		February		March	
	Average	1935	Average	1936	Average	1936	Average	1936
Temperature, ° F.								
Maximum	26	19.3	21.0	17.9	22.0	17.9	33.0	39.0
Mean	20	14.8	13.0	11.9	14.0	10.3	26.0	31.55
Minimum	13	10.4	5.0	6.0	7.0	2.72	18.0	24.11
Total precipitation	3.74	1.245	3.75	3.27	3.17	3.05	3.52	4.41
Snowfall, in.	23.7	9.75	28.5	28.5	24.4	25.5	24.4	11.25

Average figures taken from Canada Year Book for 1931. (Total precipitation includes snowfall calculated at 10 in. snow = 1 in. rain.)

Discussion

Up to the present, few data have been available on the lethal effect of Canadian winter temperatures on the non-parasitic stages of the common helminth parasites of sheep while on pasture land. It has been possible, from the information available on the bionomics of certain parasites, to predict the improbability of their survival throughout the winter. Undoubtedly the survival of helminths would not depend merely on one factor such as temperature, but on a complex of factors, such as precipitation, period of exposure to a certain temperature, snow-cover, type of herbage, location of pasture and many other factors.

The small number of experimental animals employed may be criticized but the observations recorded were significant, not relative to the numbers employed, but to the fact that the animals used must have been raised without possible chance of bursate nematode infestation. *Strongyloides papillosus* was observed in most cases, and on account of the certainty with which the ovum can be identified, was disregarded throughout the experiment. It has been found practically impossible to raise lambs free of this parasite.

Chance contamination of the lambs exposed to the "pure-line" infection in each pen could possibly have occurred during the culturing and preparation of the infective larvae for drenching. This chance, however, was eliminated by the use of a control lamb in the two pens where pure line infections were attempted.

Conclusion

The following common helminths of sheep demonstrated the ability of being able to survive a normal Canadian winter and to cause an infestation within the sheep when ingested at pasture during the following spring: *Moniezia expansa*, *Ostertagia circumcincta*, *Nematodirus filicollis*, *Trichostrongylus colubriformis*.

These species were able to withstand temperatures ranging from $-15°$ F. to $52.5°$ F. encountered during the four month period.

It is apparent that pastures cannot be considered as free from these helminths after exposure to climatic factors experienced during one normal Canadian winter.

Canadian Journal of Research

Issued by The National Research Council of Canada

VOL. 15, SEC. D. SEPTEMBER, 1937 NUMBER 9

THE TAXONOMY OF *DIPHYLLOBOTHRIUM LATUM* (LINNÉ, 1758) IN WESTERN CANADA[1]

By Robert Arnold Wardle[2] and Ellen Louise McColl[3]

Abstract

On the basis of accepted criteria for the differentiation of species of *Diphyllobothrium* Cobbold, diphyllobothriid material from humans and dogs in Manitoba, one of the three endemic areas of diphyllobothriosis in North America, is adjudged to be homogeneous and to be specifically identical with authenticated *D. latum* material from Russia, Finland and Japan. The classificatory value of various characteristics of *D. latum* and the extent of variability of these characteristics in Canadian material are discussed.

Introduction

In spite of a long and intimate sociological association, the physiological differences between man and dog are sufficiently profound to discourage the common possession of identical species of parasites. *Diphyllobothrium latum* (Linné, 1758) is, with the possible and doubtful exception of *Dipylidium caninum*, the only tapeworm found commonly and habitually in both dog and man, and if we except a few forms of restricted distribution that stray to man occasionally from the dog, it is the only parasite, external or internal, common to both dog and man and apparently equally at home in either host.

It is exceptional also among tapeworms in its wide range of definitive hosts, a range that includes at least seven species of *Felis*, four species of *Canis*, four species of seal, three of bear, one of whale, mongoose, fox, mink, domestic pig and man.

Such catholicity of host adoption, when shown by a member of a group of parasites notoriously restricted in their choice of definitive hosts, should raise the suspicion that the characters chosen arbitrarily for the delineation of this species are inadequate, and that *latum* is in fact not a species at all but a group of closely related physiological species in course of evolutionary divergence. The point has not been raised previously, to our knowledge, and no close comparison appears to have been made between alleged *latum* specimens from different host species within a restricted area.

Relatively common in sled dogs in certain districts of Manitoba is a form of tapeworm sufficiently resembling the accepted descriptions of *latum* to justify identification with it (4, 5, 9, 12). It is not uncommon also among

[1] *Manuscript received June 17, 1937.*
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ranch-raised foxes and mink. To what extent it occurs among wolves, bears and wild foxes is undetermined; nor is its distribution among the human population accurately known. Fecal examinations of hospital patients, university students, reservation Indians, and so forth, suggest an extremely low incidence of infestation, probably not exceeding 2%. Apart from people of Indian blood, the population is predominantly French, Scottish, Ukrainian, Icelandic and Jewish in ancestral origin, and the consumption of raw fish is not a common custom. A few cases of human diphyllobothriosis are noted each year, however, by medical practitioners, chiefly among Jewish housewives, and there is reason to attribute some of the cases recorded in other cities of North America to initial infection from fishes of Manitoba origin.

Taxonomic Criteria

The validity and limitations of the characters used in distinguishing species of tapeworms have been discussed previously (10). For the present comparison the characters selected as being least likely to be influenced by variations of the host environment were the uterine shape, the position of the common genital pore, and the "height", *i.e.*, the dorso-ventral measurements in a 16μ-thick transverse section of a gravid proglottis, of the cuticle, subcuticle, cortex, vitellaria, muscle zone, medulla, testes and cirrus sac, as compared with the height of the proglottis itself. Observations were also made upon the shape and size of the scolex, strobila and proglottids.

The material, comprising specimens from humans in Manitoba, northern Russia, Finland and Japan, and from dogs in Manitoba and northern Russia, had been fixed in formalin, and for purposes of microscopical examination was stained by Delafield's haematoxylin technique, dehydrated in dioxan, cleared in methyl salicylate and mounted in xylol-balsam.

Scolex and Strobila

The scolex of *Diphyllobothrium latum* is described by European authors as spoon-shaped (spatulate), almond-shaped (amygdalate) or olive-shaped (olivate), and approximately 2 mm. long by 1 mm. in maximum breadth.

The shape and size of the scolex and strobila of a living tapeworm just removed from the host and observed in an artificial medium are continually altering owing to the absence of skeletal tissue. The extent of the change is determined by the chemical nature, hydrogen ion concentration and temperature of the medium, and by the length of time that the tapeworm has been divorced from its host. This question has already been discussed (9, 11), but the point may be stressed that even if the fixative employed were to kill the tapeworm instantaneously, which in actual practice never happens since the action of the fixative is impeded by the cuticle, no two specimens will be identical in shape and size after being killed, even if approximately so when alive. Precise descriptions of the shape and size of the scolex and strobila are therefore unreliable. On the other hand, the post-mortem shape and dimensions are determined by the extent of muscular relaxation at death,

so that, among a range of specimens killed in the same way by exposure to a weak fixative such as 5–10% formalin, it should be possible to determine the limiting boundaries of shape and size, since the extent of muscular relaxation will vary only within certain limits.

Among the specimens obtained from dogs and foxes in Manitoba,—approximately 100 specimens—the shape of the scolex varied from narrowly lanceolate to broadly spatulate (Fig. 1). A similar range of shape was seen

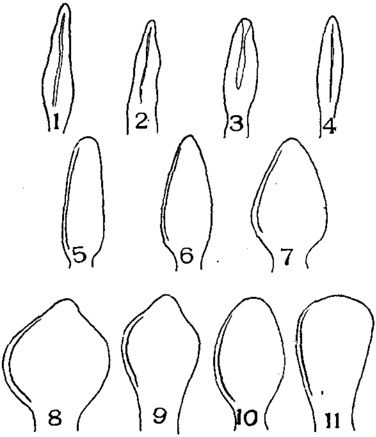

FIG. 1. *Range of scolex shape of Diphyllobothrium latum in Manitoba material. 1, 2, 3, 4 scolices less than 25 hours old; 5, digitate; 6, lanceolate; 7, ovate; 8, cordate; 9, clavate; 10, olivate; 11, spatulate.*

among collections of 20–27 scolices from individual dogs. The commonest type among local diphyllobothriid material is the clavate (Fig. 2), with its maximum width at one-third of the length from the bluntly rounded apex of the scolex. There is a tendency, probably correlated with a progressive deterioration of the scolex musculature, for the shape of the scolex to change, as the worm ages, from an active lanceolate or digitate type to a relatively inactive clavate or spatulate type.

Among 29 scolices taken from a dog infested for 75 days, the lengths varied, after formalin fixation, from 1.326–2.400 mm. with a mean value of 1.700 mm.; the breadths varied from 0.625–1.000 mm. (maximum breadth accompanying minimum length) with a mean value of 0.747 mm. Among 24 scolices from a dog infested for 56 days the mean length by breadth values were 1.523 × 0.926 mm. In one scolex from a Russian dog, infested 36 days, the shape was clavate, the dimensions 1.25 × 0.75 mm. Unfortunately only

four scolices from man were available. Two Russian scolices were spatulate, with mean dimensions of 1.5 × 0.75 mm. Two scolices from man in Manitoba were clavate with mean dimensions 1.25 × 0.9 mm. Magath (3) describes one scolex from man in Minnesota as 1.4 × 0.5 mm. in dimensions, and figures it as midway between digitate and olivate.

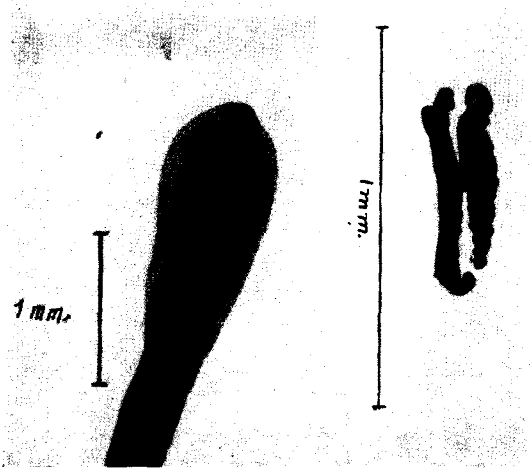

FIG. 2. *Scolex of Diphyllobothrium latum from cat, Winnipeg, seen in surficial view and in transverse section.*

The shape of 27 scolices removed from the host (kitten) within 25 hr. after initial infection was invariably acutely lanceolate (Fig. 1) and the mean dimensions 1.66 × 0.5 mm. Presumably, therefore, no growth of the scolex takes place after the plerocercoid larva reaches the definitive host. This appears to be the case in tapeworms generally. The scolex is necessary to anchor the small and muscularly weak early strobila, but in the older strobila it serves little purpose, the tapeworm maintaining its station by muscular tonus.

The neck region extends from the posterior margins of the bothria to the first interproglottid boundary. Although the most important physiological region of the tapeworm, it is histologically the most obscure, since it does not react with the usual microtechnical reagents. Its posterior margin—the first interproglottid groove—is difficult to fix with precision, so that the extent to which the neck region corresponds with the original plerocercoid body is

uncertain. Among the 27 individuals up to 25 hr. old, mentioned above, the lengths—including the scolex—varied from 6–24 mm., with a mean of 10.0 mm. Segmentation begins usually at about 25 hours after initial infection. Among five 30-hr. strobilae, whose mean length was 45 mm., the neck region varied from 3.5–4.0 mm. in length and can represent therefore only the anterior third of the plerocercoid. Among 23 specimens from a dog infested 56 days, the neck lengths varied from 1.4–2.6 mm. with a mean value of 2.2 mm. Magath (3), however, estimated the neck length of a single specimen from man as 8 mm.

Segmentation of the early strobila between 25 and 72 hr. old appears almost simultaneously along the whole strobilar length, the primary strobila being thus formed as in other families of pseudophyllidean tapeworms—Ptychobothriidae, Amphicotylidae—where no neck is present. The absence of a neck from certain diphyllobothriid species parasitic in pinnipede Carnivora and ascribed to the genus *Diphyllobothrium* Cobbold would suggest that this method of primary strobila formation was the original method in *Diphyllobothrium* also and that the neck is, from an evolutionary point of view, a new structure. It would be a matter of great interest to know whether, in such apparently primitive diphyllobothriids, the secondary strobila is formed as in Pseudophyllidea generally, by division of pre-existing proglottids or, as in *Diphyllobothrium latum*, by origin of new proglottids from the neck region.

The early strobila is completely segmented by the 72nd hour after initial infection. It consists of "long" proglottids, *i.e.*, proglottids with a length of 1.5–2.0 times the breadth. The proglottids are markedly craspedote and the terminal proglottis has a characteristic bluntly rounded posterior margin. In superinfested dogs such primary strobilae may be found several months after initial infection. The alleged species *parvum* of Stephens (7) appears almost certainly to have been a primary strobila of *latum* about nine metres in length, occurring in man, but under the conditions of light infestation such as occur usually in human hosts it is probable that the primary strobila passes fairly rapidly into the secondary phase.

The new proglottids of the secondary strobila are at first linear, much wider than long; as they mature they become quadrate; under conditions of ample nutrition they remain linear, the breadth being three to four times the length. Under conditions of superinfestation in dogs the characteristic shape of the secondary proglottids is quadrate, or nearly so, but strips of long and craspedote proglottids may interrupt the sequence of quadrate proglottids. Such recurrence of primary strobilar characters may arise from a slowing down of genital development and a release of material for tissue growth. Under conditions of light infestation and ample nutrition the proglottids are linear, because the nutritional demands of the reproductive system limit the surplus of material available for tissue growth. It is probable that a survey of tapeworms in general would show that when the number of individuals per host is habitually low, in consequence of premunition, and the sexual development rapid, the proglottid shape is usually linear.

The most extensive data concerning the size of the strobila in *D. latum* in Europe are those of Tarassow (8) who found that, among 405 specimens from humans in the Karelia-Murmansk-Leningrad districts of U.S.S.R., the strobilar length varied from one to seventeen metres, the majority of the specimens being 8-10 metres in length. Tapeworm material obtained from human hosts by vermifugation is usually incomplete, and accurate measurements of strobilae from North American human hosts are not available. Scattered observations suggest that they rarely exceed two metres in length. In dogs they are smaller still. In superinfested dogs in our experiments the mean strobilar length varied from 25.6 cm. (32 days after initial infection) to 169 cm. (98 days after initial infection).

On the whole, therefore, variations in shape and size of the scolex and strobila of *Diphyllobothrium latum*, although of considerable physiological interest, have little taxonomic value.

Internal Dimensions

There has been a tendency in recent years for students of tapeworm taxonomy to provide meticulous micrometric measurements of such internal structures as the cirrus sac, testes, germarium, vitellaria and genital ducts, and exhaustively detailed descriptions of the cuticle, subcuticle, musculature, parenchyma and so forth. Valuable as such ponderous and scholarly descriptions must be to the student of comparative histology, it may be doubted whether they are of like value to the taxonomist, although certainly preferable to the inadequate descriptions that have accompanied the announcement of many alleged new species. A tapeworm has no system of skeletal tissues such as serve in other animals to keep the dimensions of anatomical structures between definable limits, and such structures as the cirrus sac, uterus, and genital ducts have markedly contractile muscular walls that make them extremely susceptible to the distorting effects of technical reagents. A tapeworm killed quickly by suspension in a hot aqueous medium such as 10% formalin or corrosive sublimate-acetic acid, or even hot water alone, will increase in length by 50–60% owing to the relaxation of the longitudinal musculature. Absolute dimensional measurements of tapeworm structures are therefore of doubtful value unless the material under comparison has received identical treatment. On the other hand, relative measurements where the length or breadth or height of the proglottis is used as a standard of comparison have considerable value when applied to a range of material killed and preserved in like fashion.

The data in Table I are mean values of the height, *i.e.*, the vertical diametrical measurements, of the cuticle, subcuticle, cortex, vitellaria, muscle zone, medulla, testes and cirrus sac, expressed as percentages of the height of the proglottis measured at a point midway between the middle line and the lateral margin, except in the case of the cirrus sac whose height is compared with the height of the proglottis in the middle line.

TABLE I

DORSO-VENTRAL DIMENSIONS OF PROGLOTTIS STRUCTURES EXPRESSED AS PERCENTAGES OF THE PROGLOTTIS THICKNESS. LENGTH, BREADTH AND THICKNESS OF THE PROGLOTTIS ARE GIVEN IN MILLIMETRES

Origin	Lgth.	Bdth.	Th.	Cut.	Sc.	Cort.	Vit.	Musc.	Med.	Cs.	Test.
Man, Russia	4.18	10.0	0.700	0.7	4.0	24.0	14.0	7.0	30.0	0.66	21.0
Man, Finland	2.90	8.0	.760	0.4	8.0	14.0	11.0	16.0	21.0	.47	15.0
Man, Japan	3.22	10.0	.661	1.06	6.8	15.0	10.6	16.0	22.7	.43	16.4
Mean valnes				0.72	6.2	18.0	12.0	13.0	24.6	.52	17.5
Man, Winnipeg.	2.47	7.5	.856	0.94	6.0	23.5	9.4	11.7	17.6	.50	14.0
Man, Winnipeg	3.54	10.0	.590	0.34	8.5	11.0	13.6	22.0	33.2	.51	24.6
Man. Winnipeg	1.97	7.0	.174	0.28	7.0	16.7	9.8	15.4	21.0	.57	18.1
Mean values				0.52	7.2	17.1	11.0	16.3	24.0	.53	19.0
Dog, Russia	5.15	5.0	.850	1.17	4.7	27.5	11.7	11.7	17.6	.75	17.6
Dog, Churchill	1.05	3.0	.978	1.40	12.0	14.3	12.2	14.3	21.1	.31	13.8
Dog, Winnipeg	7.87	6.5	.540	2.80	11.1	14.8	11.1	3.7	22.2	.60	14.0
Mean values				1.79	9.3	19.5	11.0	9.9	20.3	.55	15.1

Cut. cuticle, Sc. subcuticle, Cort. cortex, Vit. vitellaria, Musc. muscle zone, Med. medulla, Cs. cirrus sac, Test. testes.

The material compared comprises three specimens from man in Manitoba, three from man in Leningrad, Finland and Japan, and three specimens from dogs in Manitoba (Winnipeg, Churchill) and Leningrad. The material in each case was fixed in formalin, embedded in paraffin, cut to a thickness of 16μ and stained by the iron-haematoxylin technique.

Comparison of data from human material suggests that, despite considerable individual variation in the percentage proportions of the internal structures measured, there is a fundamental similarity, brought out particularly by comparing the mean values of the three foreign specimens with those of the Manitoba specimens. The chief differences are in thickness of cuticle, which might be expected to vary with host gut conditions, and thickness of the muscle zone, which might be expected to vary with the degree of relaxation induced by fixation. Among the specimens from dogs there was greater variation, especially as regards the cuticle, subcuticle and muscle

zones, but again the mean values of the three do not depart so widely from those of human material as to suggest any specific differentiation. The material from Churchill, the most northerly location for *D. latum* yet recorded in Canada, consisted of twelve strobilae, believed to be primary, with a mean ength of 30 cm. and width of 3 mm. This material showed certain peculiarties, notably the markedly digitate scolices, 1.7 × 0.7 mm. in dimensions, he great proglottid thickness (978μ), extremely thick subcuticle (90μ), hick muscle zone (140μ), small cirrus sac, small testes—scarcely larger than he vitellaria—, with long axes vertical instead of horizontal, and the absence of circum-cloacal papillae which are usually very prominent in material from logs. In the absence of human material from this area, however, it would e premature to draw any conclusions as to specific or sub-specific distinction oncerning this material.

Genital Pore

The descriptions of the genitalia of *D. latum* provided by early authors have een incorporated and added to in the accounts given by Sommer and andois (6) and by Magath (3) and need not be repeated here.

The term "porus genitalis" is not clearly defined by earlier authors and ctually in *latum* there are three such pores, the vagina and cirrus sac opening eparately into a genital cloaca which itself has an exit to the exterior. The

TABLE II

OOP COUNTS, EACH SIDE OF THE MIDDLE LINE, FOR THE ANTERIOR UTERUS (Ant. ut.) AND POSTERIOR UTERUS (Post. ut.) AND RATIOS OF DISTANCE (Gp.) OF COMMON GENITAL PORE FROM ANTERIOR PROGLOTTID MARGIN TO PROGLOTTID LENGTH (Lgth.). THE VALUES ARE MEAN FOR *n* CONSECUTIVE PROGLOTTIDS

Origin	*n*.	Ant. ut.	Post ut.	Lgth., mm.	Gp., mm.	Ratio
an,						
Russia	20	5.2 : 4.7	2.05 : 2.55	5.67	1.35	0.234
Russia	20	5.9 : 5.5	2.80 : 3.20	4.18	0.857	.205
Finland	20	6.3 : 6.0	1.80 : 2.30	2.90	0.417	.144
Japan	20	4.7 : 4.3	1.40 : 2.00	3.22	0.825	.256
Manitoba	20	4.2 : 4.5	1.30 : 1.50	2.47	0.399	.162
Manitoba	10	4.1 : 4.4	2.40 : 3.40	3.54	0.810	.229
Manitoba	20	4.2 : 4.5	2.70 : 2.90	1.97	0.386	.196
Manitoba	20	3.8 : 3.4	1.30 : 2.00	1.70	0.308	.181
Manitoba	20	3.9 : 3.4	1.10 : 1.70	1.38	0.252	.182
Manitoba	20	4.9 : 5.1	1.90 : 2.20	2.07	0.330	.159
Manitoba	20	4.9 : 5.2	2.00 : 2.50	3.03	0.635	.209
Manitoba	20	5.0 : 4.7	— —	1.70	0.312	.184
g,						
Russia	20	7.2 : 7.3	2.9 : 2.7	5.15	1.210	.235
Gimli	12	5.6 : 6.3	1.5 : 1.3	2.30	0.308	.134
Winnipeg	7	8.0 : 7.9	3.1 : 3.9	7.87	2.012	.255
Winnipeg	12	6.2 : 6.0	2.8 : 2.4	1.60	0.324	.202
Churchill	9	4.0 : 4.0	— —	1.05	0.184	.175
r						
Gimli	20	5.3 : 5.6	2.7 : 2.6	3.06	0.572	.187

term will be used here to imply the central point of the common genital pore or cloacal aperture. This is frequently placed vertically ventral to the male pore but occasionally, due to distortion of the cuticle, it is shifted to the female pore region. Its position is usually described in terms of its distance from the anterior margin of the proglottis and is a favorite taxonomic character with students of Diphyllobothriidae. In European specimens it is usually said to be situated at about the anterior quarter of the proglottid length. In the case of a Minnesota specimen Magath (3) gives a distance of 0.44 mm. from the anterior margin of a segment 2.5 mm. in length, that is to say a ratio to proglottid length of 0.18.

Table II gives this ratio of genital pore position for a series of consecutive proglottids, usually 20, in material from man in Russia, Finland, Japan, Manitoba (eight specimens) and from dogs in Russia and Manitoba (Winnipeg, Gimli, Churchill). In the material as a whole the ratio varied from 0.134 (Gimli dog) to 0.255 (Winnipeg dog) with a general mean of 0.196. In Manitoba human material the ratio varied from 0.162–0.229 with a mean of 0.188; in Manitoba dog material it varied from 0.134–0.255 with a mean of 0.19. The material from a fox had a ratio of 0.187; from a dog at Churchill, a mean ratio of 0.175. On the whole therefore the common genital pore in Manitoba material is nearer the anterior margin of the proglottis than it is in Russian or Japanese material, but the difference of position is too slight to constitute a valid specific differentiation.

Uterine Shape

The uterus of *Diphyllobothrium latum* is a tube fixed between its entrance from the oötype and its exit to the exterior, and thrown into a series of coils or "loops". The number of these loops on each side of the middle line has long been a taxonomic character for species of *Diphyllobothrium*. The steps in the development of this looped condition have not to our knowledge been described and may be briefly discussed here.

In a specimen from man in Leningrad, the anlage of the uterus first appears at 50 mm. from the scolex tip, that is to say between the 250th and 300th proglottids. In the next 156 proglottids, a distance of 165 mm., the uterus appears as a *straight, faintly staining structure*, presumably tubular, with the anterior end swollen and bent ventrad upon itself (Fig. 3, A) although the apparent swollen extremity may be the anlage of the cirrus sac. By elongation through growth, the straight uterus becomes a loose corkscrew which persists through the next 84 proglottids (Fig. 3, B). Continued growth brings about a lengthening of the corkscrew coils, producing a zigzag pattern that persists through the next 80 proglottids (Fig. 3, C). As eggs begin to pass from the oötype into the uterine tube the somewhat angular bends become rounded, closer together, loop-like and approximately parallel, but directed slightly dorsad and ventrad from the horizontal plane. A distinction now appears between an anterior set of loops—large, approximately parallel, non-stainable, containing eggs, each with a single, longitudinal

wrinkle—and a posterior set of loops—small, irregularly arranged, stainable with haematoxylin, containing eggs whose shell, in mounted specimens, is usually intact or occasionally in a condition of general collapse. The wrinkling of the eggs is due to dehydration by the reagents used in microtechnical preparation. In weak formalin or in water no such wrinkling is shown by extruded eggs; in alcohol of 70% or greater strength, a single longitudinal wrinkle appears immediately. Eggs in the posterior uterus appear to have no operculum, whereas those in the anterior uterus are operculated; the distortion of the egg shell observed among the latter eggs seems in some way to be correlated with the presence of the operculum.

The loops of the posterior uterus are difficult to count, often distinguishable only with difficulty, and although used by Faust *et al.* (1), are commonly ignored by systematists.

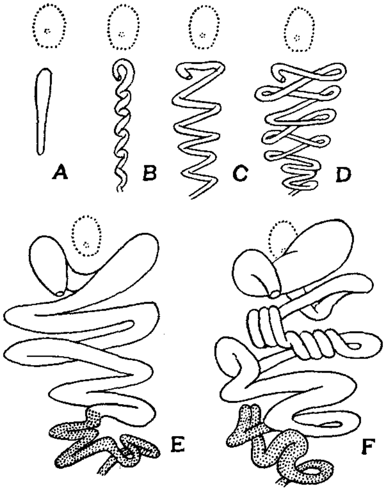

FIG. 3. *Stages in the development of the uterus of Diphyllobothrium latum. The shaded area indicates the posterior uterine region.*

The primary uterine pattern is thus a series of parallel and lateral loops, whose anterior members have thin, chitinoid and non-stainable walls enclosing eggs wrinkled longitudinally (in mounted specimens), and whose posterior members have thicker, epithelioid, stainable walls enclosing eggs that are either not distorted or else occasionally in a condition of general collapse. If this primary pattern persisted through the whole strobila the uterine loop

count would be a relatively stable and easily estimated taxonomic character. Unfortunately, the difficulty of loop counting is greatly increased by egg elimination—exhausted proglottids are almost impossible to use for loop counts—,and by distortion induced by egg pressure within the uterus.

In the primitive, long type of proglottis, the anterior loop count is approximately eight on either side of the middle line, the posterior count approximately three or four on either side. However, the later quadrate and linear proglottids show a reduction from these numbers, and their uterine loops, owing to the intrinsic pressure of the increasing number of eggs, aided by the extrinsic pressure of the muscular medullary margin, lose their parallel alignment and lie at an angle to the central area so that a rosette-like arrangement of the loops is produced. This is especially marked in the posterior uterine region. The bends of the loops become bloated with eggs, and in mounted preparations may give the appearance of a linear series of egg sacs on either side of the middle line. The two most anterior bends are particularly enlarged. Continued pressure brings about a corkscrew-like torsion of some of the anterior loops, or a sub-looping, so that in flattened preparations they may appear forked. Russian material shows this pseudo-forking to a marked degree; Manitoba material shows more of the torsion effect. If a fork be counted as two loops, then the loop counts of European material will appear greater than those of Manitoba material. The use of uterine loop counts as a taxonomic character is therefore not altogether satisfactory. The count is difficult to estimate with accuracy and the values found vary within relatively wide limits.

Anterior and posterior loop counts—each the mean of 20 consecutive proglottids—are given in Table II for material from various sources. It will be noted that the Manitoba human material agrees fairly well in having approximately 4.3 anterior loops and approximately two posterior loops on each side. The single Japanese specimen agreed on the whole with the Manitoba material. The Russian and Finnish material, owing to the prevalence of forked loops, showed higher mean counts of 5.6 for anterior and 2.5 for posterior loops. Material from dogs, both Russian and Manitoban, showed higher mean counts, the mean being 6.5 for anterior, 2.6 for posterior. For the material as a whole, excluding the Churchill dog material, the mean anterior count was 5.3, the posterior count 2.3.

The influence of secondary distortion upon the uterine loop counts is shown in Table III which gives the results of loop counts of 150 consecutive proglottids of Russian human material and 70 consecutive proglottids of Manitoba human material. To condense the table, only the means of each set of ten proglottids are given. It will be noted that in Russian material where secondary pseudo-forking is marked and the forks are counted as single loops, the uterine loop counts increase apparently from 3.3 : 3.2 in the anterior uterus, and 1.2 : 1.16 in the posterior uterus, to 5.5 : 5.2 : in the anterior uterus and 2.0 : 2.7 in the posterior uterus respectively, whereas in Manitoba material, where such pseudo-forking is rare and distortion produces chiefly

TABLE III

Uterine loop counts for consecutive proglottids of the one strobila. The values represent means of successive sets of ten proglottids

Russian (human)		Manitoba (human)	
Anterior uterus	Posterior uterus	Anterior uterus	Posterior uterus
3.3 : 3.2	1.2 : 1.6	3.4 : 3.1	0.5 : 0.8
3.0 : 3.0	1.4 : 1.3	3.2 : 3.5	0.8 : 0.6
3.3 : 3.5	1.6 : 1.9	3.3 : 3.2	0.7 : 0.9
4.2 : 4.2	1.9 : 1.9	3.0 : 3.3	0.2 : 0.7
3.9 : 3.8	1.6 : 2.2	3.6 : 4.1	0.9 : 1.0
4.2 : 4.6	1.9 : 1.7	3.8 : 3.7	0.7 : 1.0
4.4 : 4.3	1.7 : 2.0	3.7 : 3.5	1.1 : 0.7
4.5 : 4.5	1.5 : 2.2		
4.3 : 4.5	1.9 : 2.4		
5.0 : 4.9	1.8 : 2.4		
5.2 : 4.6	1.5 : 2.5		
5.3 : 5.8	1.9 : 2.2		
5.2 : 5.5	2.3 : 2.0		
5.6 : 5.3	2.0 : 2.1		
5.5 : 5.3	2.0 : 2.7		

spiral twisting of the loops, the anterior uterine counts vary only between 3.4 : 3.1 and 3.7 : 3.5, the posterior uterine counts vary only between 0.5 : 0.8 and 0.7 : 1.0.

Conclusions

If we accept as valid criteria of specific distinction the characteristics of *Diphyllobothrium latum* discussed above, there does not appear to be sufficient difference between Manitoba human and canine material, Manitoba and Russian human material, Manitoba and Russian canine material, to suggest that more than one species is involved. There are however certain slight but constant differences between Manitoba and foreign material, and the Manitoba material may be defined as: *Diphyllobothrium* sp., probably *latum*, with scolex lanceolate to spatulate in shape but commonly clavate; with proglottids linear in human material but quadrate or even longitudinal in canine material; the dorso-ventral dimensions of the internal structures, expressed as percentages of the proglottid height are for the cuticle 0.34–2.8 (thickest in dogs), sub-cuticle 3.0–11.1, cortex 11.0–23.5, vitellaria 9.4–13.6, muscle zone 9.6–22.0 (thinnest in dogs), medulla 17.6–33.2, testes 14.0–24.6, cirrus sac (percentage of *maximum* proglottid height) 0.50–0.57. The position of the common genital pore, measured from its centre to the anterior proglottid margin, varies between 0.134–0.209 of the proglottid length. The uterine loop counts vary from 3.4–4.9 on either side, with a mean value of 4.3, for the anterior loops, and from 1.1–3.4 with a mean value of 2.0 for the posterior loops, in human material; in canine material the counts are 5.3–8.0 for the anterior loops, 1.3–3.9 for the posterior loops. Circumcloacal papillae absent or faintly visible in human material, prominent in canine material.

On the whole the Manitoba material shows somewhat closer resemblances to Japanese material than to European, but the limited amount of foreign material available for comparison precludes any definite conclusions to this effect.

Acknowledgments

The authors must express grateful thanks to Dr. D. Nicholson, Professor of Pathology, University of Manitoba, for the loan of an extensive collection of diphyllobothriid material from man and dog in Winnipeg; to Dr. G. K. Petruschewsky, of the Fischereiinstitut in Leningrad, for material from man and dog in northern U.S.S.R.; to Dr. T. B. Magath of the Mayo Clinic, Rochester, Minnesota, for material from man in Finland and Japan; to Dr. R. H. Buller, F.R.S. and the National Research Council of Canada for financial assistance.

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PLASMA LIPIDS IN ACTIVELY IMMUNIZED RABBITS[1]

By Eldon M. Boyd[2], J. H. Orr[3] and G. B. Reed[4]

Abstract

No statistically significant change was noted in the phospholipid and free cholesterol content of the plasma of 16 rabbits following a period of six weeks of active immunization against *Streptococcus viridans* as compared with 28 non-immunized controls.

Boyd (2) has shown that during fever and infection in man there occurs a decrease in the concentration of plasma lipids, to which reaction he has applied the term, lipopenia of fever. Investigating this reaction, Boyd, Orr and Reed (4) found that normal variations of body temperature were not accompanied by significant alterations in the percentage of plasma phospholipid and free cholesterol in healthy rabbits. From this and other evidence it appeared that variations in plasma lipid values during fever and infection were not due to the changes in body temperature *per se*. In the present study an attempt was made to determine whether the development of antigenic reactions is a causal factor in producing changes in lipid metabolism during fever and infection. Velluz (7) was unable to demonstrate any effect of immunization upon serum cholesterol, and Felton and Kauffmann (5) found no change in the alcohol-soluble material of blood, which may be taken as a rough index of the total lipid. Hyman (7), however, reported slight decreases in both phospholipid and free cholesterol in a brief report of work in which apparently little attention was given to variations which might have occurred in control animals.

The procedure in the present investigation was as follows. Sixteen healthy rabbits were actively immunized against a strain of *Streptococcus viridans* which had been isolated from a human case of endocarditis. The animals were injected on three successive days with increasing standardized doses of a vaccine prepared from the heat-killed organisms. This procedure was repeated 10 days later with larger doses and a third series of living organisms was given after a second 10-day interval. This procedure effectively produced active immunity. The agglutination titre of serum rose from an average of less than 1 in 10 before to 1 in 3200 after immunization, and the opsonic index rose from 1 to 3.2. None of the animals died or became seriously sick when the living organisms were injected. The organism was known to be actively pathogenic to rabbits because when injected into rabbits not previously immunized it invariably produced a marked infection and a high mortality rate.

Twenty-eight other rabbits were used as non-immunized controls. There occurred no significant change in the serum content of agglutinins or opsonins in the control group. In neither the control nor immunized group did any appreciable alteration in the rectal temperature occur.

[1] *Original manuscript received July 5, 1937.*
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Blood for lipid analysis was taken after starving the animals for 15 hours, one specimen being obtained before and one after immunization, and at corresponding periods in the control animals. The samples of blood were heparinized and alcohol-ether extracts were prepared by the method of cold dilution (3). The phospholipid and free cholesterol content of these extracts were determined by the oxidative micromethods of Bloor as modified by Boyd (1).

The results obtained have been statistically summarized in Table I. Values reported include the average value before immunization, the average after, the average difference, and the standard deviation of the average difference

TABLE I

EFFECT OF ACTIVE IMMUNIZATION UPON THE PHOSPHOLIPID AND FREE CHOLESTEROL CONTENT OF RABBIT PLASMA

(The results are expressed in mg. per 100 cc. of plasma.)

Group	No. of animals	Average phospholipid				Average free cholesterol			
		Before	After	Difference	Standard deviation of difference	Before	After	Difference	Standard deviation of difference
Immunized	16	71	54	−17	22	16	14	−2	12
Non-immunized	28	70	75	+ 5	23	19	19	0	7

calculated by a formula previously given (1). Presented in this manner, a difference to be significant should be at least twice its standard deviation. It may be seen from the table that the difference, neither with phospholipid nor with free cholesterol, was sufficiently great to satisfy the requirements of this criterion of significance. There was practically no change in the free cholesterol content before and after immunization, and the slight decrease in the means for phospholipid was not great enough to be statistically significant. Hence it may be concluded that following a period of six weeks of active immunization of rabbits with *Streptococcus viridans* there is no significant change in the serum content of phospholipid and free cholesterol. Since variations in the concentration of one lipid in plasma seldom occur without corresponding changes in the concentration of other lipids, it is probable that the immunization as performed would not have produced any changes in the concentration of any lipid in the plasma of rabbits. Keeping in mind that the experiments were performed only on one species and with one organism, they suggest that the changes in the concentration of plasma lipids during fever and infection are not due to the processes concerned with the production of active immunity.

The abstract given at the beginning of this paper may be taken as a summary of the results obtained.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 15, SEC. D. OCTOBER, 1937 NUMBER 10

LARVAL AND ADULT STAGES OF BRITISH COLUMBIA ANOMURA[1]

BY JOSEPHINE F. L. HART[2]

Abstract

The external morphology of the adult stage of four species of Anomura (hermit crabs), *Upogebia pugettensis* (Dana), *Paguristes turgidus* (Stimpson), *Pagurus beringanus* (Benedict) and *Orthopagurus schmitti* (Stevens) is compared and the larval stages described. Emphasis is placed on the value of larval stages in clarifying our understanding of the taxonomy and interrelationships of the group. This study seems especially important in the Anomura because of the modifications of the adults which fit them for so specialized a mode of existence. It seems probable that some of the similarities exhibited by them are due more to convergence, as a result of similar mode of life, than to close affinity. This is illustrated by the superficial similarity of the adults of *Paguristes turgidus* and *Pagurus beringanus* and the dissimilarity of their larvae, as contrasted with apparent differences between the adult *Pagurus beringanus* and *Orthopagurus schmitti*, whose larvae are alike. By a comparison of the least modified appendages (the mouth parts) of the adults, the same relationship becomes apparent as was suggested by a study of the larvae. It is therefore concluded that by a comparison of both the larval and adult phases a clearer understanding of the affinities of the species under consideration is obtained.

Introduction

The classification of decapod Crustacea has been long a subject of controversy. One of the chief reasons for dispute is that the animals have been classified almost entirely by a consideration of adult characters. Thus it is to be expected that studies of the larval phases might clarify our understanding of the taxonomy of the group and perhaps suggest relationships not now appreciated. For instance, Lebour (23) has proposed a rearrangement of the classification of the British Brachyura based on a study of the larvae. A study of development would perhaps be even more illuminating in such a group as the Anomura, because of the modifications which fit them for so specialized a mode of existence. It is possible that some of the similarities which the adults exhibit are due to convergence as a result of a similar habit of life. Only ontogenetic studies can conclusively elucidate such a question.

The present study concerns four species of Anomura from British Columbia: *Upogebia pugettensis* (Dana), *Paguristes turgidus* (Stimpson), *Pagurus beringanus* (Benedict) and *Orthopagurus schmitti* (Stevens). The conclusions drawn as to the taxonomy of these forms are based on studies of the developmental stages as well as on the adult characters. Comparatively little

[1] *Manuscript received August 11, 1937.*
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information is available on the larval stages of Anomura and none of the forms considered here has been previously described. Except for characters used in identification, the morphology of the adults has received little attention.

Upogebia pugettensis is a symmetrical Thalassinid, not as greatly modified for its burrowing existence as many other members of the group, but with thin integument and lamellate pleopods, which are correlated with this mode of life. The other three species, however, do show modifications correlated with their life in spiral shells or in a straight tube. *Pagurus beringanus* and *Paguristes turgidus* live in gastropod shells and have twisted abdomens and asymmetrical tail-fans, while *Orthopagurus schmitti* lives in a worm tube and shows a lack of bilateral symmetry only in the difference in size of the chelae, and in the distribution of the pleopods. These species were not chosen haphazardly. *Paguristes turgidus* has a number of unusual structures in the adult and its larvae are quite unlike the described larvae of *Pagurus*. *Pagurus beringanus*, on the other hand, is typical in both larval and adult stages, and as its habitat is similar to *Paguristes*, seemed suitable for comparison. The third hermit crab, *Orthopagurus schmitti*, is of interest because of its greater abdominal symmetry and the form of its larvae. *Upogebia pugettensis* was chosen because it lives under somewhat the same conditions as *Orthopagurus* and exhibits a number of apparently primitive characters in all phases.

History of the Study of Classification

The classification of decapod Crustacea has been the subject of much discussion during the last hundred years and continues to be a difficult problem. In 1806, Latreille divided the order Decapoda into Macrura and Brachyura. The division was based on the type of abdomen:— in the first group, well developed and carried straight out behind, and in the second, turned forward under the thorax. This grouping, however, does not hold in the case of some families in which the abdomen is carried in the same manner as in the crabs or Brachyura, but which are obviously more closely allied to the long tailed Macrura. H. Milne-Edwards in 1834 introduced "Anomura", for a group intermediate between the other two and including the higher Macrura and lower Brachyura of Latreille's system. The members of this new group were those which had abdomens somewhat modified from the primitive macrurous type and still showed some remnants of a locomotive function and, with the exception of the Homolidae and Raninidae, retained paired appendages on the sixth segments. These two families are now usually placed in the Brachyura, but because of the type of larvae found in those species whose life history has been investigated, it seems probable that they will soon be moved again from the Brachyura. With the addition of some of the higher Macrura, the Anomura is now considered a natural group. In confirmation of this idea, Gurney (16) has found that the larvae of members of the group have at least one feature in common: the reduction in size of one pair of marginal setae on the telson.

Boas (6) pointed out that the divisions, Macrura, Anomura and Brachyura were not comparable nor were they of the same status taxonomically. His contention was that the Macrura consisted of a great variety of forms, some of which were more closely allied to members of the Brachyura or of the Anomura than they were to other members of their group. He therefore divided the order into the suborders Natantia or swimming forms, comprising the freely swimming shrimps, and the suborder Reptantia or creeping forms, including the Brachyura, the Anomura, the Thalassinidea, the Astacura, the Palinura and the deep-sea Eryonidea. Borradaile (10) adopted the main points of Boas' classification. His (9) most important change in this connection was the inclusion of the Thalassinidea (sand shrimps) with the tribe Anomura (hermit crabs), because of their resemblances to the primitive symmetrical hermit crabs. According to Borradaile, the only constant important differences are the presence in the latter of a pleurobranch on the last pereiopod, a suture across the telson, no epipodites on the legs, a reduced rostrum, subchelate fourth legs, and narrow uropods.

Benedict (3, 4) revised the nomenclature of the genera of the Paguridae and replaced *Eupagurus* with *Pagurus* and suggested *Pagurias* for those Pagurids described during the nineteenth century as *Pagurus*. These changes do not seem to have been universally adopted, but as recent American authors have used them in the revised form, *Pagurus* in this paper refers to those formerly called *Eupagurus*. This use, however, makes the names Pagurinae and Eupagurinae given in Borradaile's (10) classification untenable and therefore the subfamilies are referred to here as I and II.

Class *CRUSTACEA*

Order DECAPODA.

Suborder *Reptantia.*

Tribe Anomura.

Superfamily Thalassinidea

Family Callianassidae

Subfamily Upogebiinae

Upogebia pugettensis (Dana)

Superfamily Paguridea

Family Paguridae

Subfamily I

Paguristes turgidus (Stimpson)

Subfamily II

Pagurus beringanus (Benedict)

Orthopagurus schmitti (Stevens)

Food

The feeding habits of all the species concerned in this paper are somewhat similar. McGinitie (26) says that *Upogebia pugettensis* feeds on detritus, which is caught by means of the "basket" formed by long hairs on the first two pairs of pereiopods through which water, containing particles of plant and animal remains, is forced by the action of the pleopods. The detritus is then cleaned off the "basket" with the third maxillipeds and conveyed by the other appendages to the mouth. If large particles of food are introduced, they are rejected by the animal.

No work has been done on the feeding habits of the hermit crabs of British Columbia, but as the habitat and modifications of the mouth parts are similar to those species that have been studied, it would seem safe to assume that they are alike in this respect. Orton (34, p. 920) states "*E. bernhardus* obtains food largely by its small claw and third maxillipeds, and may at certain periods of the year feed mainly on micro-organisms, and at others on macroscopic, but mainly tiny living organisms which have been disturbed and captured in the deposits on the sea bottom. Food can be obtained by the use of the third maxillipeds alone. The mouth parts of *E. bernhardus* are not adapted for rapid ingestion of the meats of animals in bulk, and the gastric mill is less strongly developed than in the carnivorous crabs: these facts, along with certain features of the gut, indicate morphological and anatomical modification correlated with the food and mode of feeding."

Orthopagurus schmitti, owing to its enforced stationary existence, is a plankton feeder, catching the organisms in the water by means of the action of its third maxillipeds, which are fringed with stiff bristles. The mandibles of all these species are comparatively feeble, indicating that the food does not require much mastication.

Material

The study of decapod Crustacea, on which the present paper is based, was commenced in the summer of 1928, when a collection of shore species was made in the vicinity of Victoria, British Columbia. The results of the study of this collection, together with some additional specimens obtained the following summer at Nanaimo, were published in 1930 (18). Specimens of adult Anomura, obtained while the writer was working at the Pacific Biological Station, Nanaimo, from 1929 to 1934, have been augmented by specimens from Victoria, and from points on the west coast of Vancouver Island, collected by Mr. E. G. Hart in 1934. Thus a considerable number of individuals of the species dealt with here were available for study.

Records of *Orthopagurus schmitti* have been published only by Stevens (40, 41), who described the species from Friday Harbor, Washington, and by the writer (18) from Nanaimo. *Upogebia pugettensis* is the only one of the species dealt with in which the morphology has been investigated except for those features used in the classification. The descriptions of the structure of the other three species are therefore given here for the first time.

Knowledge of the life history of decapods is scanty, especially concerning species from the Pacific coast of North America. From this area Berkeley (5) has published descriptions of some of the larval stages of five pandalid shrimps, and of five Hippolytidae (Berkeley (5) Needler (31)). Marukawa (27) described the larvae of the Anomuran King crab, *Paralithodes camtschatica* (Tilesius). All the larval stages of four species of true crabs were reared from the egg, and described by the writer (19). Thus the Anomuran larvae of British Columbia, with the exception cited above, are unknown.

The experimental rearing of Brachyura and Anomura was therefore started during the summer of 1930 (May until August) and continued during the same period of 1931, from January until July in 1933 and during July and August, 1934. Three species of hermit crabs were reared from the egg to the adolescent stages during 1931 and 1934 by the writer. In addition to these complete series, more than two dozen incomplete life histories, comprising about 75 post-embryonic stages, have been obtained by rearing from the egg. The culture methods have been described briefly (20, 21).

One soon becomes familiar with the characters of various groups and even of some of the genera, so that the stages can be readily distinguished in the plankton. The larval stages of allied forms are often very similar, and specific differences are difficult to determine. In a genus such as *Pagurus*, with some 15 species in British Columbia, careful and detailed comparison of the larvae is necessary, in order to determine specific features with any degree of certainty, and rearing from the egg is essential. The spawning season of most species is prolonged, so that the number of species to be found in the plankton at any time is great. The larvae are usually distinctively colored, but as all color is lost in preserving them, morphological differences must be relied upon. In the Anomura, the characters showing the greatest variation among species, and yet the least modification during the development and growth of the individual, are the carapace, the abdomen, the antennae and the telson.

Morphology of Adults

Upogebia pugettensis (Dana)

Upogebia pugettensis (Dana), the so-called mud-shrimp, is the first species dealt with here. There has been considerable controversy in the past about the position in the system of classification of the group to which this species belongs. The Thalassinidea are shrimp-like burrowing Reptantia. Correlated with their mode of life are certain modifications, such as a reduction in the number of gills, softening of the integument of the abdomen, and reduction in size of the pleura. It would seem probable that some of the characters in common within the group are due to convergence and not to close phylogenetic relationship. The Thalassinidea resemble the hermit crabs in some respects and the lobsters in others. Features common to both Thalassinidea and the lobsters, are the shape of the tail-fan, of the first leg

and often of the second. They resemble the hermit crabs in the mode of concealment, in the thorn-like shape of the antennal scale (where this structure is present in the adult), in the freedom of the last thoracic sternite and the mode of carrying the last pair of legs. In view of these features, Borradaile (8) and Calman (12) put the family in the tribe Anomura, while Ortmann (32) and Bouvier (11) consider them to be more closely allied to the lobsters. Gurney (16), from a study of the larvae, feels that the resemblances to the Anomura are greater in this phase. He also points out that the Thalassinidea is not a homogeneous group, and that with further knowledge it will be split into at least two series. We are, however, concerned here only with one species of the subfamily Upogebiinae and its resemblance to the hermit crabs, and in this connection the Anomuran affinities are of greatest interest.

Stevens (42) has ably described and figured this species and made ecological notes. The ecology has also been studied by McGinitie (26). The papers of these two writers have been most useful in the discussion and comparison of the species dealt with in this paper.

It will perhaps suffice to mention that *Upogebia pugettensis* (Dana) is macrurous in shape, often reaching 90 mm. in length of body, and dwells in burrows, about 25 mm. in diameter, tunnelled in sandy mud or gravel by means of the animal's walking legs and tail-fan. The species occurs commonly on tide flats from Southern Alaska to Lower California.

Paguristes turgidus (Stimpson)

Paguristes turgidus (Stimpson) is a hermit crab inhabiting fairly deep water (to 254 fathoms) from British Columbia to Southern California. It usually occurs on a rocky bottom and in considerable abundance. The shell commonly occupied is the moon shell, *Polinices*, and there is a tendency for the individual to choose a shell larger than necessary, so that it can withdraw itself completely within its shell and can be reached only by breaking the shell. Although this is the only species of the genus recorded from British Columbia, other species are found in California and in nearly all tropical and semi-tropical regions.

P. turgidus may have a carapace of about 25 mm. in length, with a total body length of 75 mm. It is an interesting form because the chelae are subequal, the left being the larger; paired appendages in addition to the uropods are present on the abdomen and there is an egg pouch in the female. The animal is in general a deep red, toned down in color by tan hirsuteness and the brown bases of spines. The antennules and eyestalks are striped longitudinally with red and white. The abdomen is yellow, except in the mature female, where the ova show scarlet through the thin integument. The pleopods are pink and the uropods red with yellow setae.

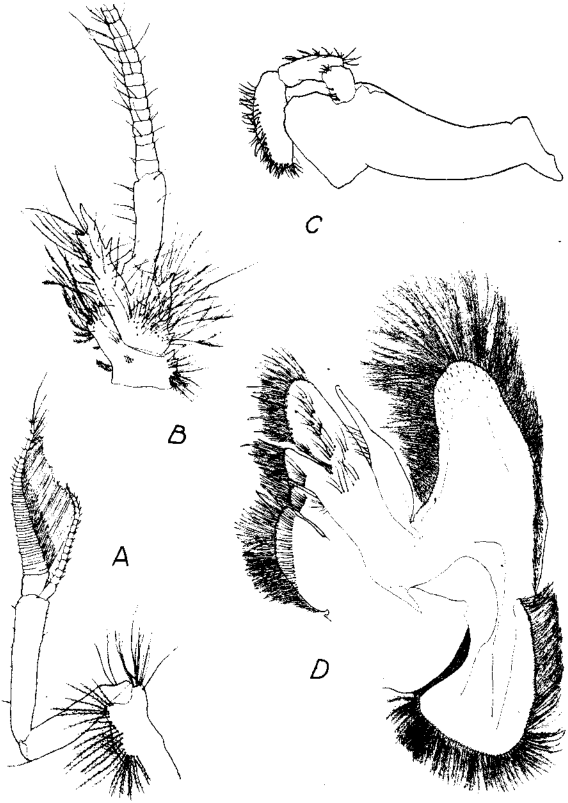


FIG. 1. *Paguristes turgidus (Stimpson). Male. A. Antennule. B. Antenna. C. Mandible. D. Maxilla.*

Female (Plate I, Fig. 1; Fig. 3, A-C)

Paguristes turgidus (Plate I, Fig. 1) is a stout species: hairy, and with dark-tipped spines on the subequal chelae and walking legs. Most of the hairs are plumose. Carapace thinly calcified, especially the posterior part. Rostrum and lateral spines subequal: small and sharp-pointed. Abdomen large, dextrally twisted, with tergum and sternum of first segment calcified; as is area around pleopods on left side and corresponding region of right side, of second to fifth segments. Sixth segment and telson also calcified. Some indication of calcification along ventral margin of outgrowth (which serves as a partial egg pouch) found on posterior part of fourth segment on left side. Telson (Fig. 3, A) asymmetrical, left side much larger than right, with sutures cutting off triangular postero-lateral portions: posterior margins toothed. Eyestalks long, cylindrical, and scales cut into a number of deep teeth.

Antennule rather flattened laterally; the first segment of peduncle with three large spines, the second and third segments narrower than first. Inner flagellum large, multiarticulate, and nearly twice as long as narrow outer flagellum. Antenna has short coxopodite; basipodite armed with a large spinous protuberance, about half the size of spined scale (exopodite): first segment of endopodite has a large spine, second a smaller one and third smooth and equal to sum of lengths of other two; flagellum comparatively short; stout and hairy.

Right mandible with a somewhat triangular incisor and a rounded molar; small basipodite; first segment of endopodite with a few setae and second with numerous bristles. Left mandible similar, except that cutting surface is rounded, with a few flat teeth.

Maxillule with endite of coxopodite broad and that of basipodite narrower and longer: endopodite rather large, with bristles on tip and large lateral and posterior extension (second segment); exite (epipodite ?) large. Maxilla has large endites, heavily bristled: a fair-sized endopodite, tapering towards tip, with sparse lateral bristles; scaphognathite large with dense brush of setae on margin, and a fold indicating fusion of exopodite and epipodite.

First maxilliped with a thick triangular coxopodite, and a flat basipodite produced distally, with stout bristles on inner margin. Endopodite composed of a single curved segment; proximal portion of exopodite swollen, heavily armed with hairs, and bearing a narrow segment with flagellum of a few poorly defined segments: and epipodite large and lying against exopodite. Second maxilliped of usual type, with endopodite of five segments and a well developed exopodite with flagellum. Third maxilliped stout with bases of appendage closely approximated: endopodite armed with a row of heavy spines on ventro-medial margin of merus, other segments with stiff bristles; exopodite large and flagellate. Two arthrobranchs: phyllobranchiate with rather elongated leaves.

First pereiopod chelate, left subequal to right; rather short, stout and heavily armed with spines and plumose hairs, except on medial surface and

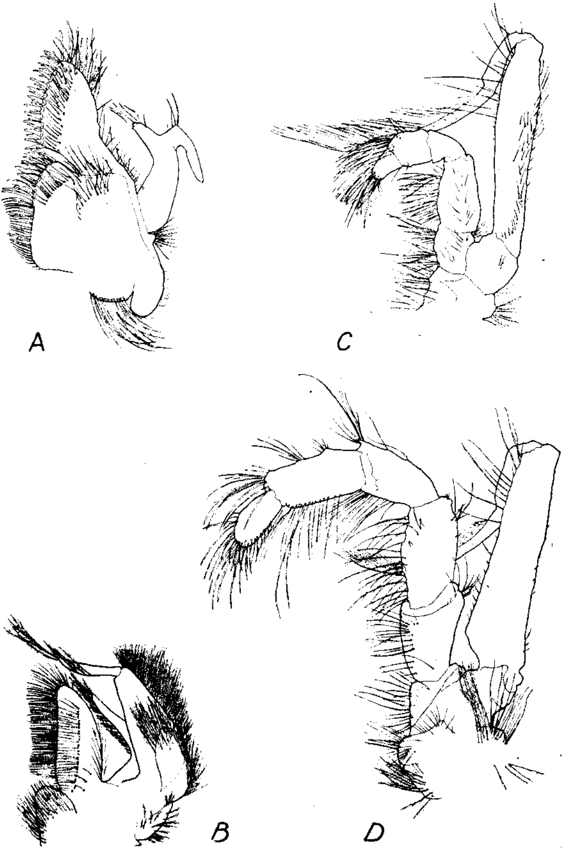


FIG. 2. *Paguristes turgidus (Stimpson). Male. A. Maxillule. B. First maxilliped. C. Second maxilliped. D. Third maxilliped.*

outer side of merus; tips of chela strongly sclerotized. Two arthrobranchs. Second and third pereiopods well developed, elongated and armed with spines and plumose hairs. Each has two arthrobranchs and one pleurobranch. Fourth pereiopod small, with long hairs and suctorial setae on distal ventral surface of propodus. It is simple, not subchelate. Two arthrobranchs and one pleurobranch. Fifth pereiopod, which is carried folded up posterior and dorsal to carapace, also small, with bunches of hairs and definitely chelate (broad flat thumb and movable finger) with suctorial setae. No gills.

First pleopod (Fig. 3, B) small, with fused joint. Distal part probably endopoditic in origin, because in *Upogebia* a similar pleopod carries eggs, and these are borne only by endopodites. Few marginal setae. Second pleopod (Fig. 3, C), as well as third, fourth, and fifth, borne only on left side. Second made up of protopodite, rather broad distally, and subequal endopodite and exopodite. Third similar, except that exopodite larger than endopodite. Fourth has an even larger exopodite than third. Fifth pleopod has no endopodite, but exopodite well developed. Uropods or appendages of sixth segment (Fig. 3, A) unequal in size: that of the left side much larger than right, and both with exopodites larger than endopodites. The flattened posterior surfaces bear numerous suctorial setae.

Male (Plate I, Fig. 2; Fig. 1; Fig. 2; Fig. 3, D-E)

Male of *Paguristes turgidus* (Pl. I, Fig. 2) differs from female mainly in armature of abdomen and its appendages. Abdomen of male more strongly calcified than that of female. First segment is similar to that of female. Tergum and part of sternum of second calcified, as is a large part of tergum of third segment, which is armed with soft hairs posteriorly. Fourth and fifth like third, but to a lesser degree, while sixth has tergum strongly calcified.

Paired abdominal appendages of first and second segments highly specialized for transference of spermatophores to body of female. Paired appendages (Fig. 3, D) of first segment lie fairly close together; each is a short, stout, uniramous limb of two segments. Protopodite margined on inner edge with stout setae and endopodite(?) hollowed out, twisted and armed with stout bristles and teeth to form upper half of tube. Second pair of pleopods (Fig. 3, E) longer and their bases farther apart with protopodite unarmed and distal half of endopodite(?) grooved to fit over that of first appendage. Second pair of pleopods covered with coarse bristles distally. Remaining pleopods (present on left side only) all lack endopodites (with one exception, a small endopodite on third pleopod of large male figured). In lacking endopodites, male differs from female of species and from males of *Pagurus* and *Orthopagurus*. Pleopods of third and fifth segments subequal in length and that of fourth somewhat longer. They all bear tufts of setae on protopodite, and exopodites are margined with numerous long plumose setae.

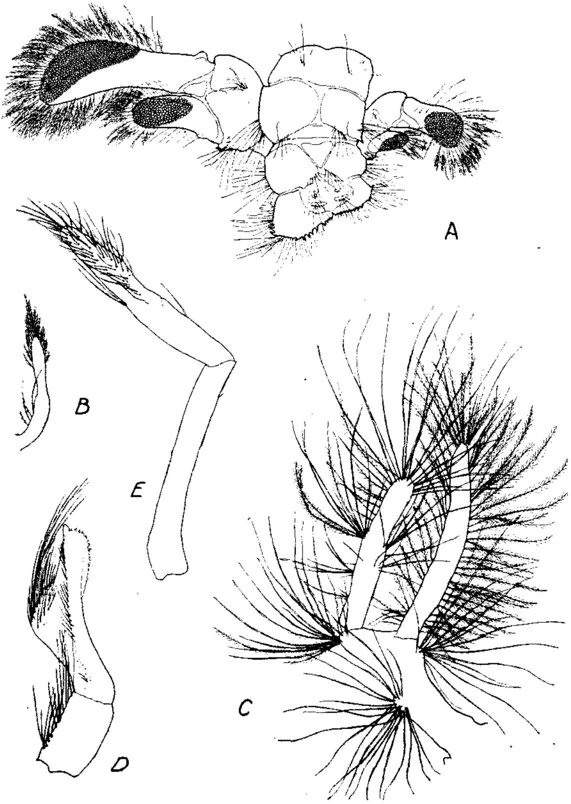


FIG. 3. *Paguristes turgidus (Stimpson). A-C, Female. A. Telson and uropods. B. First pleopod. C. Second pleopod. D, E, Male. D. First pleopod. E. Second pleopod.*

Pagurus beringanus (Benedict)

Pagurus beringanus (Benedict) is a typical hermit crab, with large right claw and asymmetrical abdomen with paired appendages on the sixth segment only. In the Nanaimo region, *P. beringanus* is commonly taken with *Paguristes turgidus*, and for this reason is used here. It is, however, a shallow water species, not having yet been obtained from water more than 40 fathoms deep. In the Nanaimo area, the adults are not found near the shore at low tide, but they are common on all rocky shores in the Straits of Juan de Fuca. The lower temperature of the water in the latter area may account for this difference in distribution.

P. beringanus usually occupies the empty shells of the whelk, *Thais* and, like Paguristes, chooses large ones. Frequently there is a break in the shell near the tip, and a small individual of the same species lives in the upper part, with a large one in the lower. The coloring is brilliant: gray-green chelipeds, except for a white band near the tips, with orange granules and spines; the pereiopods green and white, spotted with rust, which is concentrated into a band at each joint; and the remainder of the body flecked with scarlet and brown; mainly scarlet on the branchial region of the carapace and on the abdomen. The eyestalks and antennae are brown.

Female (Plate I, Fig. 3; Fig. 4, A-C)

Pagurus beringanus (Plate I, Fig. 3) is quite a stout species, with a fair number of fine hairs (none plumose). Chelipeds armed with short spines and spiny granules. Carapace may reach a length of 20 mm. and body about 50 mm. Carapace fairly well calcified anteriorly, but thin and soft posteriorly. Rostrum and lateral teeth very obtuse. Abdomen with calcified area on tergum of first segment; two lateral, elongated patches on second and third segments, and small round areas on fourth and fifth. No brood pouch. Sixth segment and telson (Fig. 4, A) similar to those of *Paguristes turgidus*. Eyestalks long, but scale small and triangular and not cut into teeth.

Antennule like that of *Paguristes* except that there are two rows of long bristles instead of scattered short setae on inner flagellum. Antenna is a narrower appendage with spines on basipodite, with a narrow sickle-shaped exopodite and a longer flagellum, with very short bristles between articulations.

Mandibles each have three blunt teeth and the separation between basipodite and endopodite is not clearly defined. Endites of maxillule not as well developed as those of *Paguristes;* endopodite differs in having only one bristle on inner side of flattened apex and a small knob on outer corner. Exite much smaller. Maxilla very like that of *Paguristes* except that there are bristles at tip of endopodite, and no suture which might indicate that the scaphognathite is made up of two parts, as is seen in the other form.

First maxilliped (Fig. 4, C) much smaller and less twisted than that of *Paguristes*. No epipodite. Second maxilliped similar in the two forms but

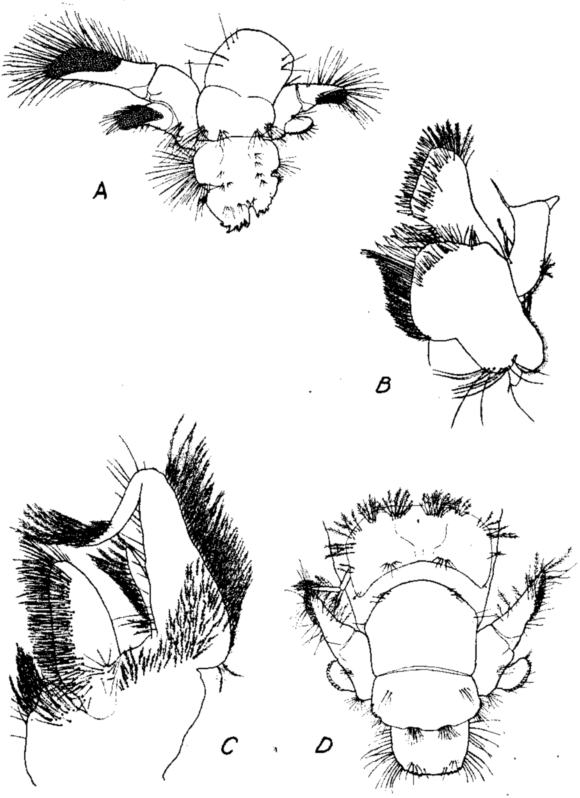


FIG. 4. *Pagurus beringanus* (Benedict). *A-C*, *Female*. *A*. *Telson and uropods*. *B*. *Maxillule*. *C*. *First maxilliped*. *D*. *Orthopagurus schmitti* (*Stevens*). *Male*. *Telson and uropods*.

flagellum slightly shorter. Third maxilliped is smaller than that of *Paguristes*, but like that species has a row of teeth on inner edge of ischium, and there are a larger number of comb setae on dactylus and propodus, in fact the whole endopodite is more densely armed with bristles. Coxae separated. Two arthrobranchs on each appendage.

Right cheliped much larger than left, and both with rather short thumbs and fixed fingers. Two large tubercles present on ventro-distal part of merus of right one. Two arthrobranchs. Next two pereiopods long, with no pleurobranchs. Fourth very small, about $\frac{2}{3}$ that of *Paguristes*, subchelate with tuberculate edge on short propodus. Two arthrobranchs and one pleurobranch. Fifth leg also small, chelate, although not as clearly so as in *Paguristes*.

No pleopods on first segment of abdomen; and those of second, third and fourth segments differ from *Paguristes*, in that the bases of exopodite and endopodite are close together, and the end of protopodite narrow. The two parts are more nearly equal in size. Fifth pleopod has a short endopodite. Uropods similar to those of *Paguristes*.

Male

Integument of abdomen of male is like that of female in degree of calcification. No pleopods on either side of first and second segments, and only on left side of third, fourth and fifth. Pleopods have a large exopodite and a small endopodite.

Orthopagurus schmitti (Stevens)

Orthopagurus schmitti (Stevens) is a small hermit crab (up to 40 mm. in length) inhabiting empty worm tubes of two species of *Serpula* and of *Sabellaria cementarium* Moore. The species is of interest here because the abdomen is straight, not coiled as in *Pagurus* and *Paguristes*, and the telson and uropods are symmetrical, but the other abdominal appendages are on the left side only as in *Pagurus*. It occurs in shallow water (to a depth of about 100 fathoms) and is obtained by dredging up the rocks to which the worm tubes are attached. At minus tides, some of these rocks are exposed at Horswell point, but in other places dredging is necessary. It is quite common locally, but has been recorded only from the Friday Harbor and Nanaimo regions.

O. schmitti is somewhat pubescent, with brown hairs. The coloring is bright: pink or white with orange-brown bands and patches.

Female and Male (Plate I, Fig. 4; Fig. 4, D)

Carapace with a hard smooth cephalic portion, and the remainder very lightly calcified. Rostrum of a fair size, but lateral teeth small. Eyestalks fairly stout, pear-shaped, and a small triangular scale. First segment of abdomen calcified, as are small patches on either side of dorsal part of second, third and fourth segments. No columellar prominence. Fifth segment with part of tergum calcified, showing indication of an overlapping fold posteriorly and apparently used to assist in retaining a hold in tube. Sixth

segment of abdomen (Fig. 4, D) with tergum well calcified and divided very definitely by a deep groove. Telson (Fig. 4, D) quite symmetrical, with rounded lateral margins and a few shallow teeth on posterior straight margin.

Antennule like that of *Paguristes turgidus* except that there are no bristles on peduncle. Antenna like that of *Pagurus*, except that setae between articulations of flagellum longer, but shorter proportionately than those of *Paguristes*.

Mandibles, maxillae, maxillipeds and first three pairs of pereiopods resemble those of *Pagurus beringanus*. Fourth pereiopod smaller than fifth, subchelate and flattened distally. Fifth does not reach carpus of third, and is chelate. Pleopods present on left side of second to fifth segments. Endopodite and exopodite subequal except for fifth, where endopodite small; protopodite narrow. Uropods (Fig. 4, D) subequal and similar, with large exopodite and small endopodite.

Pleopods of male present only on third to fifth segments.

Comparative Morphology of the Genera

The problem is to determine those characters which are not the results of modifications due to a similar mode of life (convergence) but which remain comparatively unaffected by a change of habitat and also to find those that are vestigial.

The four species dealt with here have a somewhat comparable habitat and they exhibit certain modifications in common. They all live protected by substances not secreted by themselves, *i.e.*, hollow objects: *Upogebia* in its sand burrow, *Paguristes* and *Pagurus* in gastropod shells and *Orthopagurus* in calcareous or stony worm tubes. Thin integument is found in each, especially on the carapace and abdomen. This seems to be a character associated with life in a protected spot.

Paguristes resembles *Upogebia* in the adult phase in a number of morphological characters. The rostrum of both species is small and the surface of the carapace posterior to it, roughened. In *Upogebia*, according to McGinitie (26), this serves to hold the animal in position against the top of the burrow while the pleopods fan the water for food and for aeration. In *Paguristes*, it probably assists the animal to retain a hold in its shell and to resist efforts to dislodge it. The flagella of both species are rather short and hairy and although the adult *Upogebia* has but a vestigial scale on the antenna, some allied forms have it well developed. The endopodites of the maxillules are two-jointed and the exite (epipodite?) large. There are indications that the scaphognathite of the maxilla of both species is made up of two parts (exopodite and epipodite). The bases of the third maxillipeds are approximated. The first pair of pereiopods are alike (chelate) and subequal; the fourth pair are simple (neither chelate nor subchelate) and the fifth is chelate in *Paguristes* and often in *Upogebia*. In the females of both

species, the pleopods of the first segment are small, paired and uniramous. The males have paired pleopods on the second segment, but these are modified for copulation in *Paguristes* only. The pleopods in *Upogebia* serve as a means of creating a powerful current of water through the burrow and incidentally drawing in a supply of food, while those of *Paguristes* need only occasionally stir the water within the shell. The females of both species carry the eggs on the setae of the endopodites.

In all these characters except the last, *Paguristes* differs from *Pagurus* and *Orthopagurus*, but resembles them in its mode of life and many of the modifications that fit it for such an existence, particularly the general body shape. The symmetry or lack of symmetry of the appendages does not coincide in the three species.

The carapace of *Paguristes* has the same general shape as that of *Pagurus*, but the distribution of the hard parts (calcified chitin) and of the grooves is different. The abdomen is twisted like that of *Pagurus* (of the same habitat), but shows more vestiges of segmentation. It differs, as does *Pagurus* in this feature, from *Orthopagurus*, which has a symmetrical abdomen suited for life in straight tubes. The antennules, antennal scale, mandibles and second and third maxillipeds, are similar in the three species, but are of the unspecialized decapod type, with the exception of the antennules, which are more like those of the Brachyura, than the more primitive type found in *Upogebia*. The first pereiopod is so twisted that the chelae open horizontally and the axis of the joint between the carpus and propodus is such that an "operculum" may be formed by the chelae, thus preventing intruders from entering. *Upogebia* uses the tail-fan for blocking the entrance to its burrow. The fourth and fifth legs are reduced in size, but as there is a tendency in all Anomura for the differentiation of the fifth legs, and their specialization for cleansing purposes, only the reduction in size of the fourth legs is of interest here. In *Pagurus* and *Orthopagurus* this reduction is greater than in *Paguristes* (30% and 40% of the third legs). *Paguristes* resembles *Pagurus* and *Orthopagurus* in the absence of pleopods on the right side of the second to the fifth segments of the female and of the third to the fifth of the male. The telson and uropods are asymmetrical in *Paguristes* and *Pagurus*, but not in *Orthopagurus*.

Unique Features

Paguristes turgidus differs from *Upogebia*, *Pagurus* and *Orthopagurus* in several important characters:

1. The grooves of the carapace.

2. The presence of a greater number of gills. There are ten arthrobranchs and three pleurobranchs on each side, as compared with ten arthrobranchs only in *Upogebia*, and ten arthrobranchs and one pleurobranch in *Pagurus* and *Orthopagurus*.

3. The presence of paired appendages specialized for copulation on the first and second segments of the abdomen of the male.

4. The presence of an outgrowth or egg pouch on the left side of the fourth segment of the abdomen of the female. This structure is found only in one other genus, *Paguropsis*, which according to Alcock (1) is closely allied to *Paguristes*, and differs only in having a straight abdomen, symmetrical telson and uropods, cheliform fourth legs and unpaired appendages and egg pouch indifferently on the right or left side of the abdomen.

Orthopagurus schmitti differs from the other three species in the partial restoration of the tergum of the fifth segment of the abdomen and in the symmetrical uropods adapted for holding the body within a hollow worm tube.

History of the Study of Development

Until about a hundred years ago it was believed that all decapod crustacea developed directly, in other words, with no metamorphosis. When Thompson (43) hatched the eggs of *Cancer pagurus* and obtained the zoeae, his report on these findings was not credited by many of the scientific men of the times. Westwood insisted that "*no exception occurs to the general law of development in the Crustacea; namely that they undergo no change of form sufficiently marked to warrant the application to them of the term metamorphosis*" (2, p. xliii). This idea was deeply implanted in the minds of contemporary workers largely because of the extensive work of Rathke on the development of the river crayfish, *Astacus fluviatilis*, in which, because of the life of the adult, planktonic larval stages would not be feasible. The confusion at this time was increased by observation of female crab abdomens from the tropics, to which fully developed and recently hatched young were attached. These were believed to be land crabs (13) which naturally would be governed by the same restrictions as the crayfish.

Thompson (44) described the larvae of 15 decapods including *Pagurus*. The post-larval hermit crabs were first described as adult animals under the generic name of Glaucothoë. Müller (30) surmised their true relationship to the adults.

Culture Methods

General methods for culturing both adult and larval Anomura have been given previously (20, 21). Therefore only a few additional notes are added here.

The study of the larval development of any marine form is fraught with difficulties, which are augmented in a locality where the life histories of few of the species have been worked out. Bovard and Osterid (7) published a list of those animals from which embryological material may be obtained during the months of June, July and August at Friday Harbor, Washington. This list is useful for work in the immediate vicinity of Victoria but is quite misleading for the Nanaimo district. In the latter region, although it is nearly 100 miles north of Friday Harbor, or Victoria, hydrographical conditions are more temperate, and many species spawn weeks, or even months, earlier in the season.

Few papers give any information on the spawning of Pacific coast decapods, but some record ovigerous females, and this information is sometimes of value in the case of forms that do not carry their eggs for a long period, and those that have a definite spawning season. The recording of "ovigerous" females does not take into account the degree of development of the eggs. It has thus been necessary for the present author to make her own records of the egg-carrying and of the spawning periods of the hermit crabs of British Columbia. The approximate state of development of the eggs has also been kept.

The period of incubation of the eggs differs greatly, but seems to vary in most cases directly with the size of the adult. It was found difficult to keep berried females of *Upogebia pugettensis* alive in aquaria, and thus to determine approximately the length of time the eggs are carried. McGinitie (26) found it necessary to provide the "shrimps" with tubes to live in. It is also difficult to induce *Paguristes turgidus* to hatch its eggs in the laboratory. The female will cast off a few eggs each day so that unless they are nearly ready to hatch when obtained, they will all be shed before hatching time. If a few still remain, they are generally delicate and die without casting the prezoeal cuticle.

A minimum of exposure to light seems to be preferred by most, but except for direct sunlight, fairly bright light does not appear to be especially detrimental. They are, however, more active at night, and usually the eggs hatch at this time. As low a temperature as is practicable is advisable (15–20° C.), but the majority seem able to withstand a surprising range.

Hermit crabs as a whole do not thrive without shells and as it is necessary to be able to observe the state of the eggs from time to time, the most satisfactory method is to break a window in the shell, through which the eggs may be seen, even though the animal has retired into her shell.

The egg yolk of *Upogebia* and thus the egg mass, is a bright yellow when freshly extruded, but with the absorption of the yolk and the development of the eye pigment, the mass changes to a brownish color. The egg mass of *Paguristes turgidus* is at first a bright coral-red, changing with development to yellow. The egg mass of *Pagurus beringanus* changes from brownish black to gray. The eggs of *Orthopagurus schmitti* have not yet been seen.

In the jars containing larvae, the prevention of too abundant growth of diatoms and protozoa was accomplished by moving all the larvae, by means of a large-bore pipette, as frequently as necessary to a fresh jar of sea water and food material. The sea water used was not filtered, but care was taken not to include such forms as amphipods and jellyfish, as these will clean out a jar of larvae in an extremely short time. Unless the inner surfaces of the jars were cleaned carefully, worms and barnacles were found to have settled down and grown large enough to be a menace to the larvae.

The rearing of the larvae of hermit crabs is a long and tedious process, and the mortality is extremely high. Only by repeated attempts and by

the use of a large number of individuals, may a few be brought beyond the early stages. Failure has been complete and consistent in many species, the larvae never becoming robust enough to cast off the last embryonic cuticle. However, if sufficient berried females are available, the first larval stage may be obtained occasionally, but the emergence of second stage larvae from first is the crucial point. The post-larvae are comparatively easy to keep if they are healthy when they reach this stage. The adolescent stages of those forms that will eat minced clam muscle thrive as long as they are not crowded or underfed, under which circumstances cannibalism occurs.

Larval Development

Subfamily Upogebiinae

The larval stages of European species of *Upogebia* and *Gebiopsis* have been described by various authors. Sars (38) studied a Norwegian form, Cano (14) a Mediterranean, and Webb (46) the British species. Doubt has been expressed as to the correctness of identification of these species, but since the larvae are certainly the young of these closely allied genera, the exact species is quite immaterial for comparison with the larvae of *Upogebia pugettensis* (Dana), which is found only on the Pacific coast of North America. Gurney (16) and Menon (28) have described larvae from the plankton of New Zealand and Indian waters. *U. danai* from New Zealand and *U. pugettensis* are quite similar, while the *Upogebia* (?) sp. from India shows interesting intergradations between *Upogebia* and *Paguristes* in the larval stages, a subject which will be dealt with later. Gurney (17) describes a *Upogebia* from the Red Sea in which there is no metamorphosis; the first free living form resembles the post-larva of other species.

Gurney (16) gives the characters which distinguish the larvae of Upogebiinae and with these *U. pugettensis* agrees, except for one character, namely, the presence of a distinct unarmed palp on the mandible of the second and third larval stages. The first larval stage of this species is somewhat more advanced than *U. danai* as the pleopods are quite elongate, and can hardly be described as "minute knobs", as in the latter. The second stage is similar in having six pairs of thoracic appendages with functional exopodites, but differs by the presence of a mandibular palp. The third stage also differs from that of *U. danai* and the European forms, in the shape of the telson, which is more triangular and has the posterior margin sloping slightly inwards from the lateral to the median spines. The fourth pair of spines are not strongly developed and those lateral to these are on a level with them, not on a sloping margin. There is no lateral spine on the exopodite of the uropod as is seen in other described *Upogebia*.

Upogebia pugettensis (Dana) (Figs. 5 and 6)

At Friday Harbor, Washington, females of *Upogebia pugettensis* (Dana), bearing eggs, were reported by Stevens (42) in December, February, and in the late spring and early summer. They have been taken at Departure bay,

from January until April, and the larvae occur in the plankton from February until the end of May. The egg mass is bright yellow when freshly extruded and changes to brown when ready to hatch.

There are three larval stages before the post-larval, corresponding in this respect to some species of the genus as described by Webb (46), but the larvae of this species hatch in a slightly more advanced state of development than do the European forms. This was also found to be the case in some of the Pandalids of British Columbia (5).

First larva about 3.7 mm. in length, measured from tip of rostrum to posterior margin of telson. Transparent, with a blue tinge to body, and liver showing through carapace as an olive green organ. Yellow around eyestalks, and a red chromatophore near tip and another on proximal part of antennule. Red pigment on carapace anterior to gastric mill, as well as on proximal part of first abdominal segment and on median part of telson (two chromatophores). A branching red chromatophore on basal part of non-functional third maxilliped.

Larva (Fig. 5, A, B) of the same type as those described by Webb (46). Carapace with rounded edges posteriorly and small, sharp-pointed rostrum reaching about one-third length of antennules. Abdomen composed of five segments and deltoid telson. Posterior margin of telson indented medially with a spine, a fine hair and five plumose setae on each half. Segments of abdomen smoothly rounded posteriorly, without dorsal or lateral spines.

Tubular antennule (Fig. 6, A) quite long, with several aesthetes on inner margin and terminally, and a long plumose seta a short distance from tip. Antenna (Fig. 6, B) large: protopodite with a single spinous projection ventrally, endopodite tubular with three long plumose setae terminally, and broad exopodite terminating in a stout spine and bearing eight long and one short plumose setae on inner margin.

Mandibles (Fig. 6, C) without incisor processes, and molar surfaces cut into a number of small teeth. Maxillule (Fig. 6, D) with wide clearly defined endites on protopodite, each bearing eight spines, and the two-jointed endopodite with two plumose setae on distal portion of internal edge of first segment and six on second (four terminal and two sub-terminal). Lamellate maxilla (Fig. 6, E) with endites of protopodite bilobed: coxopodite bearing ten and four setae and basipodite five and six. Endopodite with five plumose setae, but is not clearly separated from protopodite like Brachyuran zoea, and scaphognathite narrow, with about 11 plumose hairs on margin.

First maxilliped (Fig. 6, F) provided ventrally with a number of bristles, both plumose and simple: two on coxopodite and about 11 on basipodite. Endopodite five-jointed, and segments usually with three, three, one, two and five bristles respectively on ventral distal part of segments, and fine hairs on the second, third and fifth segments dorsally. Four swimming setae on exopodite. No setae on coxa and basis of second maxilliped (Fig. 6, G) but four swimming setae on exopodite. Endopodite four-jointed, with third segment

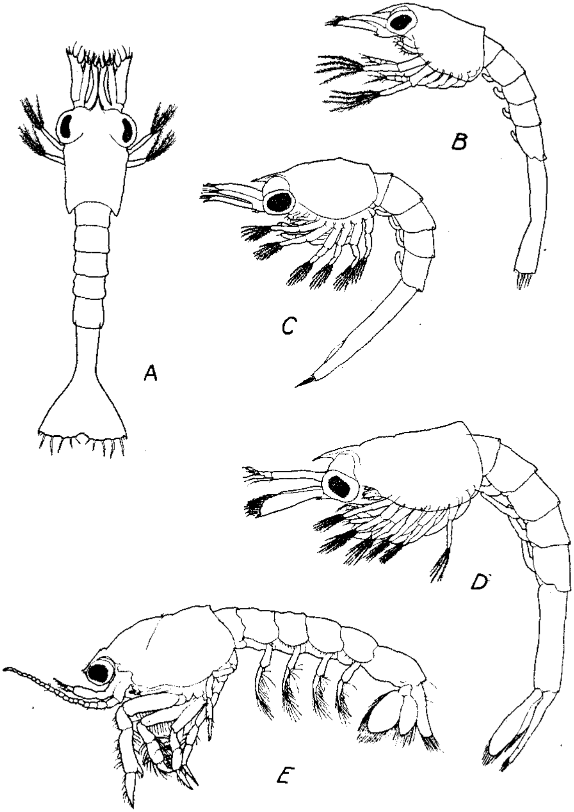


FIG. 5. *Upogebia pugettensis* (Dana). *A. First larval stage, dorsal view. B. First larval stage, lateral view. C. Second larval stage, lateral view. D. Third larval stage, lateral view. E. Post-larval stage, lateral view.*

equal in length to the other three, and segments bear one, one, two and three setae respectively. Large, non-functional exopodites present on rudimentary third maxilliped and on first three pairs of pereiopods. Endopodites on all these anlagen well developed. Four pairs of pleopods will be developed, indicated in first stage by knobs on abdomen.

Second Stage (Fig. 5, C; Fig. 6, H-L). Second larva (Fig. 5, C) about 4.4 mm. in length and similar in color to first. Sixth abdominal segment not yet separated from telson (Fig. 6, H) which is similar in shape to that of first stage, but has an extra pair of plumose setae medially and a small median tooth on posterior margin. Fourth pair of setae now considerably longer and stouter than others. Uropods show clearly through integument but still fully enclosed within telson membrane.

Antennule (Fig. 6, I) larger and tip cut into two knobs: anlagen of flagella of adult. Outer is larger and bears aesthetes, and more slender inner knob sharp-pointed, and bears fine setae. Several plumose setae on inner and a tuft of fine hairs on outer margin of peduncle. Antenna (Fig. 6, J) more elongate than that of first stage and endopodite still bears three setae. A spine on disto-lateral part of protopodite in addition to toothed one of first stage.

Now six setae on exopodite of both first and second maxillipeds. Third maxilliped (Fig. 6, L) with functional exopodite bearing six setae and a small bare endopodite which arises at proximal part of basipodite and reaches to its tip. Functional exopodites now on first, second and third pereiopods; those of the first two bearing six setae and that of the third, five. These appendages are similar to third maxilliped except that their endopodites larger; decreasing in size posteriorly. Fourth and fifth legs small and non-functional, but showing indications of segmentation. Pleopods quite large but show no subdivisions.

Third Stage (Fig. 5, D; Fig. 6, M-O). Third stage (Fig. 5, D) about 5.4 mm. in length and similar in color to the earlier stages. Abdomen now composed of six segments and telson (Fig. 6, M), which is more spade-shaped and bears three teeth on each side (with a fine hair between the two lateral), and four pairs of plumose setae medially. There is a median tooth.

Antennule (Fig. 6, N) like that of second stage but plumose setae on margin of peduncle have greatly increased in size and number. Now a swelling proximally. Antenna (Fig. 6, O) large, with endopodite equal in length to exopodite and no longer bearing terminal setae. Segmentation of flagellum of post-larva can be seen through the thin integument of endopodite for some time before ecdysis.

Palp on mandible is now quite large but rest of appendage not appreciably increased in size. Maxillule similar to that of second stage. Scaphognathite of maxilla still narrow.

Six setae on exopodite of first maxilliped and of third pereiopod and seven on rest. No indication of thumb on first pereiopod. Long and slender

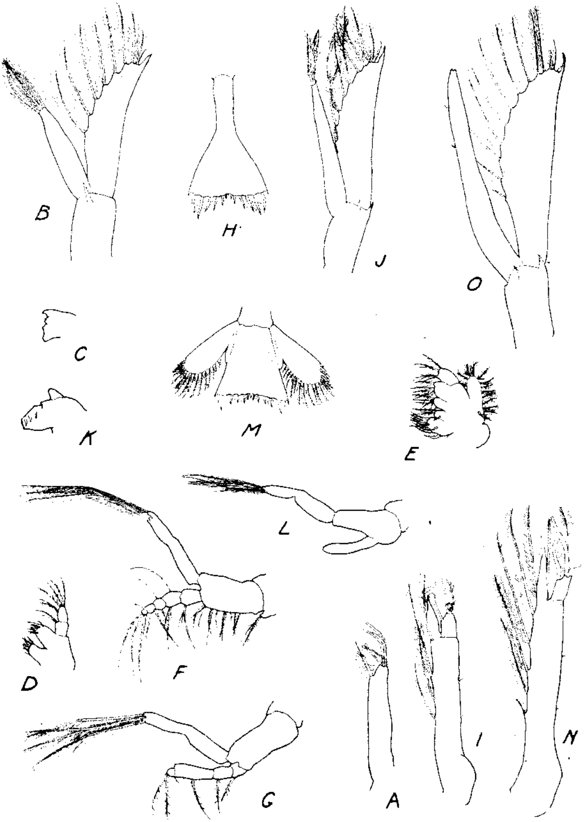


FIG. 6. *Upogebia pugettensis (Dana). First larval stage. A. Antennule. B. Antenna. C. Mandible. D. Maxillule. E. Maxilla. F. First maxilliped. G. Second maxilliped. Second larval stage. H. Telson. I. Antennule. J. Antenna. K. Mandible. L. Third maxilliped. Third larval stage. M. Telson. N. Antennule. O. Antenna.*

anlagen of pleopods show but slight indication of divisions. Uropods separate from telson: exopodite large with about 15 long plumose setae on medial margin but endopodite small and naked.

Post-larval Stage (Fig. 5, E). Post-larva about 4 mm. in length. Very transparent with a few red pigment spots, situated on anterior part of carapace, on first, second, third and sixth abdominal segments, on tips of antennules and on propodi of all the pereiopods. Post-larva (Fig. 5, E) of adult form. Obtuse rostrum with a number of bristles and small knobs distally, and lateral teeth practically obsolete. Abdomen clearly segmented but pleura narrow. Telson rectangular with long plumose setae on distal margin.

Appendages differ from those of adult mainly in clarity of segmentation and in numbers of setae. Flagella of antennule short, inner one of a single segment and outer of about three. Scale of antenna small and square. Mouth parts show no striking differences from those of adult.

A small tooth or thumb on medial distal corner of propodus of first and fifth pairs of pereiopods. Pleopods present on second to fifth segments of abdomen and of much the same proportions as adult appendages. Setae are, however, fewer in number and comparatively longer. Uropods proportionately longer and more rounded than those of adult.

GENUS *Paguristes*

Issel (22) described the larval stages of *Paguristes oculatus* Fabr. from Naples. He gives but two zoeal stages and one glaucothoe, which is a more abbreviated larval history than that of any other hermit crab yet described. It seems possible that he might have missed the normal first stage, as his description of what he considered to be the first stage has the usual second stage characteristics, with the exception of the four setae on the exopodites of the first and second maxillipeds and the non-functional third maxilliped (which, however, in his figure shows short terminal setae).

P. turgidus (Stimpson) has a short larval phase in the laboratory, reaching the post-larval in about one-sixth the average time required for other Anomura. The larvae are large when hatched and increase very little, if at all, in size during development: the post-larva is smaller than the first zoea. The integument is thin and the cast-off skins are easily missed in the culture jars.

As the time spent in each stage is so short, it seems quite probable that Issel might have missed a stage, or confused two. Dr. A. B. Needler pointed out to the writer that all first stage decapod larvae have sessile eyes, which become stalked in the second stage. Issel states quite definitely that they are stalked in his first stage larva (page 348) "i suoi occhi, relativamente piccoli, sono portati da brevissimi peduncoli." His figures do not agree in several instances with his description, *i.e.*, he puts segments in such structures as the third maxilliped so as to make an abnormal (as far as other Anomuran larvae are concerned) appendage. In several appendages he fails to separate

the dactylus from the propodus, etc. The unarmed mouth appendages are unusual but might be found in such a species that apparently does not feed in the larval phase. He figures the abdomen of the first stage as composed of seven segments and the telson, which is ridiculous, and especially so as the second stage shows five segments.

The antennule in Issel's figure of the first zoea shows the same degree of development as is found in the second zoea of *P. turgidus* and other Anomura. Also the mandible bears a palp, which is not present in the first stage of *P. turgidus* but develops in the second. The scaphognathite of the maxilla of *P. oculatus* is divided into parts in the same way as that of *P. turgidus*. The pleopods and uropods of the former are in the same state of development as those of the second stage of *P. turgidus*. The metazoea and glaucothoe of both species differ mainly in such characters as might be specific or due to inaccurate observation.

Menon (28) describes a series of larvae from Madras plankton belonging apparently to the subfamily Upogebiinae. These larvae show some interesting intergradations between *Upogebia* and *Paguristes*. The Madras form differs from both of these in having a more prolonged larval life history (five stages, instead of three or four), in the presence of epipodites on thoracic appendages 2-6, and *appendices interna* on the pleopods of the post-larval stage.

The Madras larvae resemble *Paguristes* and differ from *Upogebia* in the presence of lateral spines on the posterior margin of the fifth abdominal segment, and in the absence of a median spine on the telson until the metazoea. These two characters are the main differences between *Paguristes* and *Upogebia* in the early stages.

Paguristes turgidus (*Stimpson*) (Figs. 7, 8 and 9)

Females with freshly laid eggs (which hatched in late August and early September) were found in July near Nanaimo. To judge from the state of the eggs on specimens obtained from the west coast of Vancouver island, the spawning season there is the same as that of the east coast. The egg mass was coral red, changing to yellow as development proceeded.

There are three larval stages and probably only one post-larval. The glaucothoe did not survive long enough to moult again. The glaucothoe was obtained in the laboratory five and a half days after hatching in one instance and eight in another. *Pagurus* usually requires at least 30 days to reach the same state of development, but apparently *Paguristes* takes very little food during development and increases little in size, while *Pagurus* nearly doubles.

First stage about 4.5 mm. in length. Very transparent, with russet-red and yellow chromatophores. Chitin of antenna faintly tinged with blue, and liver yellow. Red pigment in a band anterior to liver; on carapace posterior to eyestalks, medio-laterally and marginally above maxillipeds; on abdomen laterally in paired chromatophores at junction of fourth and fifth segments and ventro-medially at bases of lateral spines of fifth segment: on

telson medially and across proximal part: on eyestalks, on bases of antennae, ventrally beneath eyestalks, at attachment of maxillae, on each mandible, on distal part of bases of first and second maxillipeds and on median part of endopodite and exopodite, and on third maxilliped. Yellow pigment medio-posteriorly on carapace; at bases of spines on second and third abdominal segments and margin of spatulate part of telson.

First stage (Fig. 7 A, B) stout. Rostrum narrow and less than one-third length of carapace, which is smoothly rounded posteriorly, resembling in this feature Thalassinid larvae rather than Pagurid. A sharp "branchiostegal" spine. Abdomen stout and dorsum of second segment produced posteriorly into a large median spine. Same regions of third, fourth and fifth segments similarly produced, but as smaller spines. Fifth segment has in addition a pair of spines on the postero-lateral margins. Telson roughly triangular and margined with seven setae on each side; the seventh (lateral) pair simple, the sixth very small and plumose, and the remainder large and plumose: fourth the longest.

Antennule (Fig. 8, A) slightly longer than rostrum, slender with one plumose seta near tip and about six aesthetes apically. Antenna (Fig. 8, B) with stout basis, narrow spinous process ventrally, and broad, flat endopodite, ending in a curved spine and bearing 12 long plumose setae on medial margin. Exopodite cylindrical, with three long plumose setae terminally.

Mandibles (Fig. 8, C) have cutting surface composed of a number of fine teeth. A slight knob on dorsal surface indicative of future palp. Maxillule (Fig, 8, D) with six plumose setae on coxopodite, two spines and two setae on basipodite and a three-jointed endopodite with one seta on distal margin of second segment and three on third. Maxilla (Fig. 8, E) with about ten setae on bilobed coxopodite, nine on basipodite, six on endopodite and ten soft plumose hairs on scaphognathite. An indication of the division of the latter into what is apparently a large exopodite and a small epipodite, covered over by a thin membrane.

First maxilliped (Fig. 8, F) composed of broad basis bearing ten bristles, exopodite with four swimming setae, and endopodite of five segments bearing respectively three, three, one, two and five setae ventrally and long fine hairs on dorsal side of second and third segments. Second maxilliped (Fig. 8, G) with three bristles on basis, four on exopodite and two, two, two and five respectively on segments of endopodite, with fine hairs on dorsal part of second segment. Third maxilliped devoid of setae and non-functional in this stage, but its exopodite reaches to end of basis of second maxilliped and endopodite about half length of exopodite.

Remaining thoracic appendages non-functional but large and well developed and hidden by margins of carapace. First and fifth pereiopods show traces of chelate tips and position of future joints can be seen in all. Fifth pereiopods medially placed and hidden under incurved anterior limbs, which are tucked up under thorax. Anlagen of pleopods not yet apparent.

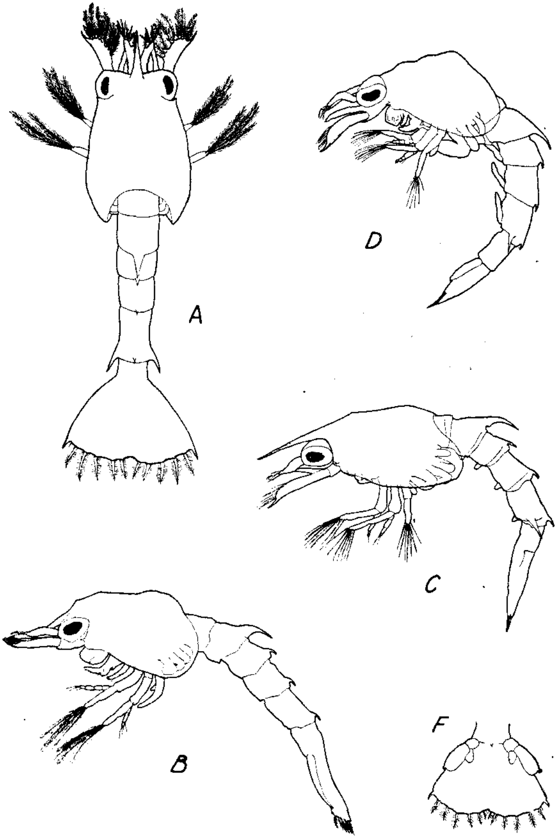


FIG. 7. *Paguristes turgidus* (*Stimpson*). *A. First larval stage, dorsal view. B. First larval stage, lateral view. C. Second larval stage, lateral view. D. Third larval stage, lateral view. F. Third larval stage, telson and uropods, ventral view.*

Second Stage (Fig. 7, C; Fig. 8, H-K). Second stage of ***Paguristes turgidus*** (Fig. 7, C) has not increased in size and its coloration is similar to first stage. Main differences are that eyes now rather elongated and stalked and that sixth segment of abdomen is separated from telson. Telson of same shape but has an extra pair of setae on medial part of posterior margin and the formation of the uropods can be seen through the thin integument. The rudimentary appendages increase in size during this stage so that it would at first glance appear that several stages were present, but there is only one more moult before the post-larval stage.

Antennule (Fig. 8, H) has increased in complexity. Plumose seta now situated on a protuberance, below which are two large plumose bristles, and aesthetes arranged in rows toward tip, which is separate from rest of appendage. Antenna not perceptibly changed.

The unusually well developed mandible (Fig. 8, I) has a naked, knob-like palp. In most pagurid species this does not appear until the last larval or post-larval stage. Maxillule similar except that the two small setae on basipodite have increased in size and are almost as large as the spines, and two additional small setae present now. Maxilla (Fig. 8, J) similar to that of first stage, except that epipoditic part is greatly increased in size and quite bare of setae and separate from exopoditic portion.

Seven setae on the exopodite of first maxilliped; about same number of setae on segments of endopodite but fine hairs of first stage replaced by long fine setae. Second maxilliped has now eight swimming setae on exopodite, and a fine seta on dorsal part of third segment of endopodite. Third maxilliped (Fig. 8, K) has exopodite functional and bearing seven setae. Endopodite quite large, comes off basis more proximally than does exopodite but not yet functional, although showing indications of segmentation.

Pereiopods large and show joints through the thin chitin. Pleopods present as distinct knobs.

Third Stage (Fig. 7, E). Third stage of ***Paguristes turgidus*** slightly smaller than second and similarly colored. Telson (Fig. 7, E) is of same shape but now two additional pairs of setae on margin medially and uropods separate.

Antennule like that of second stage but segments of appendage of following stage clearly discernible through thin integument. Endopodite of antenna now subequal in length to exopodite and a small spine distally on ventrolateral margin of protopodite.

Palp on mandible considerably larger than that of second stage. Two more small setae on basipodite of maxillule. Epipoditic part of scaphognathite longer and finger-like and bears no setae on margin but exopoditic provided with 16 soft plumose hairs.

Maxillipeds little changed except that first has now eight swimming setae on exopodite. Pereiopods well developed and segmentation of next stage clearly visible. Pleopods large: small endopodite and large exopodite. Uropods with large exopodite, terminating in a curved hook-like spine and with small endopodite.

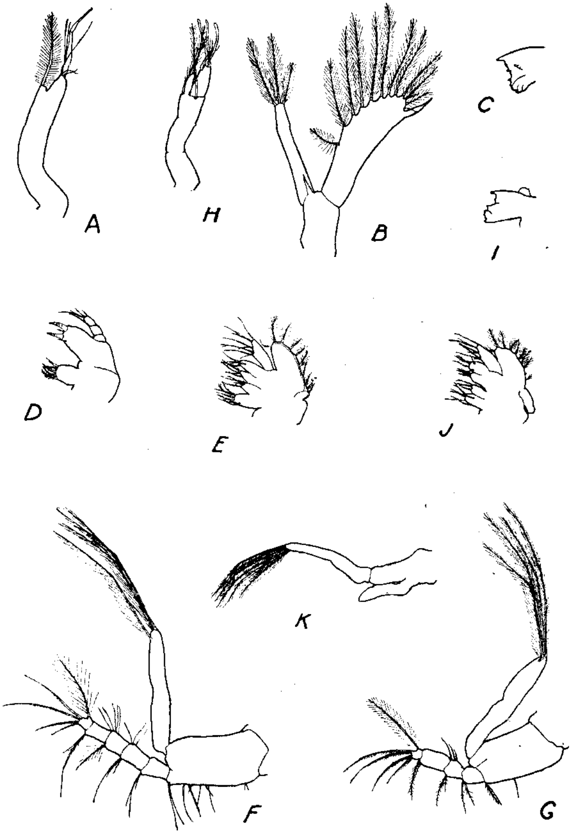


FIG. 8. *Paguristes turgidus (Stimpson). First larval stage. A. Antennule. B. Antenna. C. Mandible. D. Maxillule. E. Maxilla. F. First maxilliped. C. Second maxilliped. Second larval stage. H. Antennule. I. Mandible. J. Maxilla. K. Third maxilliped.*

Glaucothoe (Fig. 9). Glaucothoe (Fig. 9, A) about 3 mm. in total length, with carapace about 1 mm. Of the usual glaucothoe form, but with subequal chelae and only slightly asymmetrical uropods. Like the zoea, very transparent, with yellow tinged legs and russet-red chromotophores scattered over body. Red chromatophores on rostrum; on ventro-lateral edge and median dorso-posterior margin of carapace: a pair on fifth abdominal segment, numerous around margin of telson; on medial dorsal part of eyestalks, on bases of antennae, on distal part of propodi of chelae, on second and third pairs of walking legs, and on each part of uropods. Carapace with blunt rostrum. Segments of abdomen of equal length, but anterior wider than posterior. Telson hexagonal, with two lateral spines and ten long bristles distally.

Antennule (Fig. 9, B) large, with specialized setae and short bristles on first segment of peduncle; inner flagellum two-jointed and small five-jointed outer, armed with scattered bristles and aesthetes. Antenna (Fig. 9, C) has large tooth on peduncle, scimitar-shaped scale and eight-jointed flagellum, with bristles increasing in length and number toward tip.

Mandibles (Fig. 9, D) with well developed palp bearing a few short bristles on distal margin of third segment. Cutting edge rounded in right mandible and somewhat triangular in left. Maxillule (Fig. 9, E) has broad endites with short spines and bristles and endopodite of two segments, of which proximal is large and bears two bristles, and distal very small. Maxilla (Fig. 9, F) has shallow bifid endites with few bristles, finger-like endopodite, and rather narrow scaphognathite, which is divided into exopoditic and epipoditic portions by a slight fold.

First maxilliped (Fig. 9, G) with poorly developed exopodite and endopodite, almost devoid of setae, and narrow endites of protopodite with poorly developed bristles. Second maxilliped (Fig. 9, H) has large exopodite with five plumose setae on terminal segment, and five-jointed endopodite with a few small bristles distally. Third maxilliped (Fig. 9, I) also has large exopodite, with six plumose setae, and five-jointed endopodite with bristles on lateral margin and tip.

First pereiopods are chelate and subequal, with scattered spines and bristles on all segments. Second and third pereiopods long and slender. Fourth pereiopod smaller than second or third, with suctorial setae on propodus, and dactylus short, terminating in a stout claw. Fifth pereiopod also small but chelate: propodus flattened distally and flattened dactylus fits into groove of propodus; suctorial setae and long bristles on tip of appendage. Pleopods all equipped with elongate protopodites, small endopodites each with two ciccinnuli, and exopodites each bearing nine plumose setae. Uropods slightly asymmetrical and each composed of a short protopodite, with a single bristle, a stout exopodite margined with long bristles and suctorial setae, and a short endopodite similarly supplied.

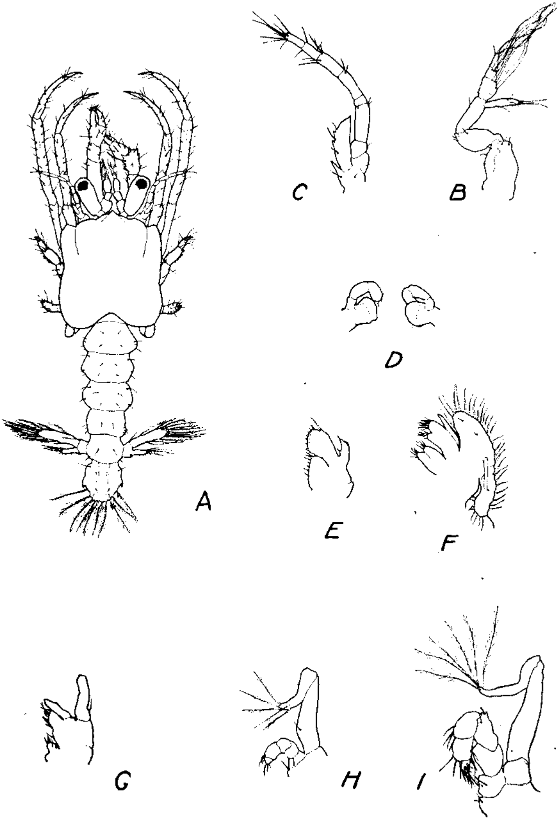


FIG. 9. *Paguristes turgidus (Stimpson). Glaucothoe. A. Dorsal view. B. Antennule. C. Antenna. D. Mandibles. E. Maxillule. F. Maxilla. G. First maxilliped. H. Second maxilliped. I. Third maxilliped.*

GENUS *Pagurus*

Rathke (36, 37) described the larval and post-larval stages of *Pagurus bernhardus.* The only important studies on *Pagurus (Eupagurus)* are by Sars (39) for the European forms and Thompson (45) for New England species. The larvae of *Pagurus beringanus* described below are very like those of *P. bernhardus* as described by Sars, and differ from those of *P. longicarpus* and *P. annulipes* by Thompson mainly in the absence of setae on the endopodite of the antenna in the first two stages.

Pagurus beringanus (*Benedict*) (Figs. 10 and 11)

Berried females of *Pagurus beringanus* (Benedict) were obtained in March, near Vancouver, and from May until August at different points off Vancouver island. The egg mass changed from brownish black to a grayish brown during the incubation period. The eggs hatched the last day in July and the first young crab was obtained in the laboratory 40 days later, after passing through four zoeal stages and one glaucothoe.

First stage (Fig. 10 A, B) about 2.4 mm. in length. Yellowish in color; rostrum, antennae, mouth parts and maxilliped exopodites blue tinged. Liver pale yellow. Red pigmentation on median anterior part of eyestalks, dorsomedially behind eyes, on carapace above bases of maxillipeds, internally around and beneath anterior part of liver, on bases of mouth parts, on bases of maxillipeds, on dorsal part of third abdominal segment, at junction of fifth segment and telson, and on anterior part of telson.

Body slender and elongate. Carapace has a long sharp-pointed rostrum about equal in length to rest of carapace and over-reaching antennae. Carapace produced posteriorly into a pair of downward curving sharp teeth. Abdomen fairly stout, of five segments, with posterior dorsal margin of second to fifth cut into four teeth, and fifth with long lateral spines in addition. Telson rather wide, triangular-shaped, and bearing seven pairs of spines and setae on posterior, medially indented margin. Lateral pair (seventh) short spines, next (sixth) minute hairs, and remainder, sharp-pointed finely serrate setae, of which the fourth is longest and rest decrease in length, five, three, two, and one respectively.

Antennule (Fig. 10, C) cylindrical, with a long plumose seta subterminally and two aesthetes at tip. Antenna (Fig. 10, D) has a short spinous process on protopodite, a narrow smooth endopodite about three-fourths length of wide exopodite, which has five plumose setae on middle third of medial margin.

Mandibles (Fig. 10, E) of usual type, with fine teeth on cutting edge. Maxillule (Fig. 10, F) has five long and two short barbed bristles on coxopodite, two toothed spines and two setae on basipodite, and no, one and three bristles on segments of endopodite. Maxilla (Fig. 10, G) lamellate, with about nine setae on both bilobed coxopodite and basipodite, seven on endopodite and five soft plumose hairs on narrow scaphognathite.

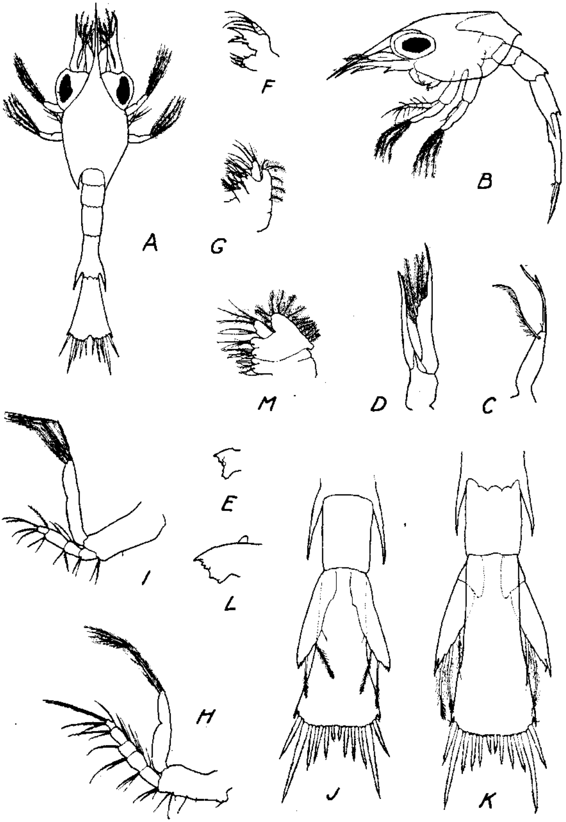


FIG. 10. *Pagurus beringanus* (Benedict). *First larval stage. A. Dorsal view. B. Lateral view. C. Antennule. D. Antenna. E. Mandible. F. Maxillule. G. Maxilla. H. First maxilliped. I. Second maxilliped. Third larval stage. J. Telson and uropods. Fourth larval stage. K. Telson and uropods. L. Mandible. M. Maxilla.*

First maxilliped (Fig. 10, H) has about nine bristles on basis, four swimming setae on exopodite and three, two, one and five respectively on ventral side of segments of endopodite and soft filaments on dorsal side of first, second and third. Second maxilliped (Fig. 10, I) has three bristles (one serrate) on basis, four on exopodite, and two, two, two and five on segments of endopodite ventrally and filaments dorsally on second and third segments. Third maxilliped non-functional: uniramous and tucked forward under thorax, between bases of other two.

Limb buds small and no signs of pleopods.

Second stage about 2.9 mm. in length and similar in color to first stage. Slight indication of separation of sixth segment of abdomen from telson, but not yet a definite somite. Telson has now an additional pair of small setae medially on posterior margin. Eyes stalked.

Antennule with plumose hair elevated on a small peduncle. Endopodite of antenna now nearly equal in length to exopodite.

Mandibles unchanged in shape. Maxillule has two more spines on basipodite. More setae (eight) on scaphognathite of maxilla than in first stage.

First maxilliped has seven plumose setae on exopodite and filaments replaced by soft plumose hairs on first, second and third segments of endopodite. Second maxilliped also has seven setae on tip of exopodite and a soft hair dorsally on second segment of endopodite. Third maxilliped has six setae on exopodite and no endopodite.

Rudimentary pereiopods slightly larger than those of first stage and appear in lateral view as four knobs covered by carapace. Pleopods not yet visible. Anlagen of uropods can be seen through thin integument of telson.

Third stage (Fig. 10, J) about 3.2 mm. in length. Now six segments in abdomen. Teeth on posterior margins of second and third segments obtuse and lateral spines of fifth segment equal in length to sixth segment. Telson (Fig. 10, J) similar except that uropods now free and fourth pair of setae almost twice as long as any of the others.

Knob on antennule increased in size and aesthetes increased in number. Antenna reaches nearly to tip of rostrum and endopodite longer and wider than exopodite. Small spine on protopodite near base of exopodite.

Mandibles and both pairs of maxillae show no appreciable change except in size.

First maxilliped has seven swimming setae on exopodite and second eight. Third maxilliped has seven and endopodite arises near base of protopodite and is small, with one terminal seta.

Pereiopods still small but tip of first one notched, indicative of chela. Fifth pair hidden under thorax and cannot be seen in a lateral view. Thickenings under integument where pleopods will appear in later stages. Uropods (Fig. 10, J) large, with tip cut into three small teeth, and three stout setae on medial margin; endopodite only a slight protuberance on medial proximal margin.

Fourth stage (Fig. 10, K-M) about 3.6 mm. in length. Differs little from third stage except in size and development of non-functioning appendages, which reach their maximum size in this stage. Sixth segment of abdomen and telson (Fig. 10, K) have elongated considerably.

Two flagella of antennule clearly defined; inner one, bare knob, with a long plumose seta at base and outer with three rows of aesthetes. Antenna similar to that of third stage.

Left mandible (Fig. 10, L) has a sharp dorsal tooth and a wide ventral one on molar process, but right composed of a number of fine teeth. Both equipped with a small bare palp. Maxilla (Fig. 10, M) has about a dozen plumose setae on scaphognathite.

Both first and second maxillipeds have eight swimming setae on exopodites. Third maxilliped has seven, and two setae on endopodite.

Chelate nature of first pereiopod now quite apparent. Pleopods long and slender, but endopoditic part not clearly defined. Exopodite of uropods (Fig. 10, L) now separate from remainder of appendage. Endopodite still just a projection of medial distal corner of protopodite.

Glaucothoe and Adolescent Crab (Fig. 11, A, B). Glaucothoe of *Pagurus beringanus* about 1.9 mm. from tip of rostrum to end of telson. Quite transparent, with yellowish tinge and scarlet and black chromatophores: black on either side of liver, and on bases of third maxillipeds, and red irregularly placed on posterior part of carapace, on second, third and sixth abdominal segments, and on propodi of chelipeds.

Glaucothoe (Fig. 11, A) small but fairly stout, with an obtuse rostrum and unarmed carapace. First segment of abdomen narrow, second wide, and rest taper to rather narrow sixth segment. Telson elongate, and subrectangular with eight terminal setae. Eyestalks large and cylindrical and their scales small and sickle-shaped.

Antennule has an elongate peduncle and inner flagellum longer than outer, which has aesthetes only on proximal half. Appendage sparsely armed with bristles. Antenna with triangular scale and flagellum of about 10 segments.

Mandibles poorly developed and small, with narrow molar portion and fair sized palp. Endites of protopodite of maxillule large, but with few and weak bristles and endopodite small and feeble. Endites of maxilla slightly bilobed and fringed with setae; endopodite small and closely applied to basipodite and scaphognathite narrow.

First maxilliped poorly developed: endites rounded and armed with a few weak bristles on margin, endopodite small and undifferentiated, and two-jointed exopodite tipped with fine hairs. Second maxilliped has a five-jointed endopodite, poorly provided with bristles and a large two-segmented exopodite ending in a brush of fine setae. Third maxilliped well developed, with a large endopodite, well provided with bristles distally and a short exopodite terminating in setae. On the whole the mouth parts are poorly developed and indicate a microplankton diet at this time.

First pereiopod chelate and toothed on cutting margins. Right larger than left. Second and third pereiopods long, slender and simple. Fourth short, somewhat subchelate, and propodus provided with a few suctorial setae. Fifth also small with short dactylus and propodus tipped with three long bristles and several suctorial setae.

Pleopods of three parts with wide protopodite, small endopodite bearing two ciccinnuli and exopodite with eight, nine, eight and six plumose setae respectively. Left uropod slightly larger than right and both composed of short protopodite, wide scimitar-shaped exopodite margined with plumose and suctorial setae and small endopodite bearing one or two setae.

Adolescent crab (Fig. 11, B) like adult except for the usual juvenile characteristics.

Orthopagurus schmitti (*Stevens*) (Fig. 11, C, D)

An ovigerous female of *Orthopagurus schmitti* (Stevens) has not yet been obtained, but on April 27, 1933, plankton was taken off Horswell point, containing metazoeae and glaucothoe of a hermit crab. These were kept in rearing jars until the adolescent stages appeared. These clearly showed the characters and coloration of *Orthopagurus*, so that it is with a fair degree of certainty of identification that these stages are compared to those of hermit crabs and sand shrimps reared from the egg.

Last zoeal stage about 7 mm. in length. Blue tinged antennae, mouth parts and pereiopods, except chelae which are yellow. A mass of scarlet pigment around liver.

Last larval stage (Fig. 11, C) of *Orthopagurus* differs from that of *Pagurus beringanus* mainly in size, color and presence of more setae on certain appendages of former. Carapace produced anteriorly into a long sharp-pointed rostrum and posteriorly into rather obtuse downward-pointing teeth. Dorsoposterior margins of third to fifth abdominal segments cut into four sharp teeth, and lateral spines of fifth segment are about half length of sixth segment. Telson (Fig. 11, D) elongate, like that of *Pagurus*, except that fourth pair of spines are only slightly longer than others.

Antennule like that of *Pagurus* except that there are two long plumose setae at base of inner flagellum, instead of one. Endopodite of antenna reaches to tip of rostrum, and is longer than exopodite, which has nine plumose setae on medial margin.

Mandibles, maxillules and maxillae very similar to those of *Pagurus* except in size.

Maxillipeds like those of *Pagurus* except that they all have eight swimming setae on their exopodites, and that there are three setae on endopodites of third maxilliped. Pereiopods and pleopods of usual degree of development for this stage. Large uropods (Fig. 11, D) nearly as long as telson, and tip of exopodite cut into several elongate teeth, while medial margin bears six plumose setae. Endopodite a small unarmed projection of protopodite.

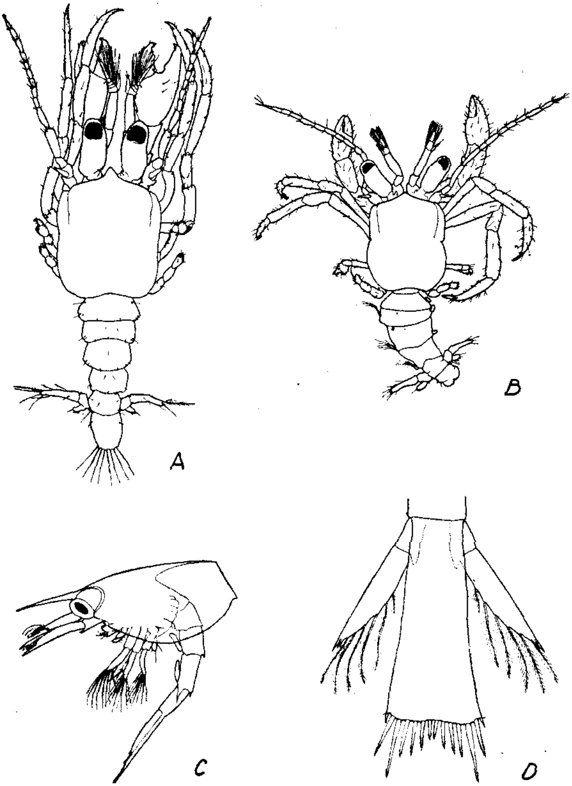


FIG. 11. *Pagurus beringanus* (*Benedict*). *A. Glaucothoe, dorsal view. B. First adolescent crab, dorsal view. Orthopagurus schmitti* (*Stevens*). *Last larval stage. C. Lateral view. D. Telson and uropods.*

Glaucothoe and Adolescent Crab. Glaucothoe resembles that of *Pagurus beringanus* except that it is nearly twice its size (about 3.5 mm. in length). A pale brown color, with fine red chromatophores on eyestalks and legs, and a large scarlet patch around gastric and heart regions.

The glaucothoe of the two species, in addition to size and coloration, also differ in the form of their chelae and tail-fans. Chelae of post-larva of *Orthopagurus schmitti* are proportionately narrower and fingers longer than those of *Pagurus beringanus*, and inner margins of carpus and propodus are toothed. Uropods longer and narrower and more heavily armed with setae. Telson elongate, with eight long setae on curved distal margin and several short bristles on each of lateral edges.

The adolescent crabs may be distinguished by their subequal uropods and coloration.

Discussion and Conclusions

The present system of classification of Anomura is based entirely on adult characters. As was pointed out by Gurney (16) ontogenetic stages should be of great value in classification and should not be neglected. The adult phase is but one stage in the cycle and therefore should not be given the importance denied another. There naturally are adaptive modifications in the larvae that present difficulties, but such convergences are equally present in the adults, so that this does not necessarily detract from the value of the larvae in a study of relationship. *Upogebia* has but a vestigial antennal scale in the adult phase, while in the larvae, the scale (exopodite), is large and well developed. It is of course essential to realize that the useful characters of the larvae are usually not those structural features used in distinguishing the adults. The conclusions drawn from the small proportion of the larvae that have been studied suggest that the larvae may be grouped by such small differences as the reduction in size of the one pair of setae on the margin of the telson or the absence of setae on the endopodite of the antennae, and these characters would appear to be at least as reliable as many of the adult features that are now considered important in the taxonomy. Unfortunately knowledge of adult morphology is in the majority of cases superficial, and is concerned mainly with the parts that exhibit obvious differences which are of value for ease of separation and of identification. The life histories and larval stages of only a small proportion of the total number of species have been investigated, but larval stages of nearly every family of Decapoda are now known, through the extensive work of Lebour and Gurney particularly. Unfortunately for the problem at hand, the Anomura have received considerably less attention than the other tribes. Hence, morphological information about all stages being scanty and specialization being well marked in the group, generalizations are as yet unsafe; evidence now available permits only tentative suggestions concerning the possible interrelationships.

In the present system of classification the genera *Paguristes* and *Pagurus* are placed in two subfamilies of the family Paguridae. Superficially these are alike, but on morphological grounds, based on both the adult and larval

phases, it would appear that their resemblances are the result of convergence due to their specialized mode of life. On the other hand, *Pagurus* and *Orthopagurus* are members of the same subfamily, and this position is confirmed by a study of their anatomy, despite the fact that on the surface they seem more unlike than *Pagurus* and *Paguristes*. *Upogebia*, placed in a different superfamily exhibits a number of characters that are considered primitive. *Paguristes*, in such features as are unmodified by the environment, shows the same primitiveness, which, however, is lacking in the other two under consideration.

In an attempt to determine genetic relationships no hint may be obtained from the study of fossil remains. Fossil Anomura are rare, only the genus *Callianassa* having been found as yet and as early as the Mesozoic era. A few specimens of recent genera, such as *Upogebia*, *Paguristes* and *Pagurus* have been described by Rathbun (35) from Tertiary and Quartenary strata, but obviously these are of no value in this connection. Ortmann (33) believes that the Pagurid group could not have originated before the late Cretaceous or early Eocene Periods. Thus relationships can only be surmised by a study of the living forms, where the problem of distinguishing between environmental and ancestral characters is a difficult one. Thompson (45, p. 197), in discussing the Paguridae, says "In fact, the relationships existing between the various genera as a whole, are very obscure. The group is in all likelihood convergent and not natural, although there is reason to believe that most of the genera are genetically related. If convergent, parts of the group may be of different ages, the characteristic mode of life being assumed at different times by various forms."

One would not expect the mouth parts to be greatly modified by the animal's mode of life, especially where feeding habits are alike, and thus any differences to be seen here should be significant in clarifying our knowledge of their affinities. The maxillules, maxillae and first maxillipeds show such features, *Paguristes* and *Upogebia* having apparently primitive characters which are lacking in *Pagurus* and *Orthopagurus*.

Borradaile (9) gives the characteristics which he considered were possessed by the forebears of the present reptant decapods. Among a number of other features which he believed primitive are a stout, curved, three-jointed palp on the mandible; the basipodite of the maxillule broader than the coxopodite and the endopodite of two segments, the second directed outwards; an epipodite on the first maxilliped; the fourth pair of legs not chelate; and many gills. *Paguristes turgidus* has retained these primitive characters which are lost in both *Pagurus beringanus* and *Orthopagurus schmitti*.

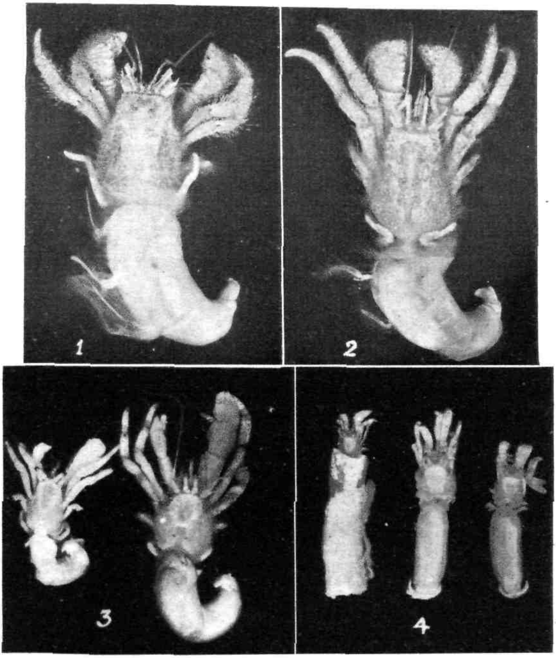
As yet few authors have used the larvae in the classification of decapods. Bouvier (11) considered the larvae in his major divisions. Gurney (16) appreciated their importance and made a number of suggestions for the revision of the systematics, based on larval characters. Lebour (23), from a study of the larval stages of the British Brachyura, pointed out affinities previously

unsuspected when the adults alone were considered. She also has made use of her knowledge of the ontogeny of various decapods in the discovery of new species. She found a previously undescribed species of *Caridion* after studying the larvae in the plankton, and in her paper (24) she quotes K. Stephensen—"but it has astonished me that the two larvae are so well distinguished from one another, for the two adult species are so closely allied that I should hardly have dared to consider them different species if their larvae were unknown." (p. 183). Recently (25) she has found another species of shrimp of the genus *Spirontocaris*, and this was also due to the discovery of a different larva in the plankton. Subsequent investigation showed two distinct types of adults among the collections previously considered to be all *S. cranchii*.

It seems probable that the relationships within the group of Anomura should be more clearly discernible among the larvae (which are all free-living components of the plankton and thus comparable) than among the adults, many of which have become highly specialized in correlation with their varied types of environment.

A study of the larvae of the Anomura with which we are concerned here, suggests the same affinities as have been proposed above in the case of the adults. The larvae of *Paguristes* resemble more those of *Upogebia* than *Pagurus*, and the larvae of *Pagurus* and *Orthopagurus* are closely allied. *Paguristes* and *Upogebia* are alike in the number of larval stages, the size on hatching, the thin integument, the stout build, the absence of teeth on the carapace, the short rostrum, and the three plumose setae on the endopodite of the antenna. *Paguristes* larvae resemble those of *Pagurus beringanus* only in the presence of lateral spines on the fifth segment of the abdomen and in the presence of only two barbed spines on the basipodite of the maxillule in the first stage. The last larval stages of *Pagurus* and *Orthopagurus* differ only in size and in number of setae on some of the appendages. One would not expect to find any trace in the larval or post-larval stages indicating that symmetry has been reacquired in the abdomen of *Orthopagurus*. It has been pointed out by Garstang (15) and by Gurney (16), that any recapitulation of such a modification of the ancestral form would be abnormal, as the larvae of the most asymmetrical forms have as typical an abdomen as do those which are bilaterally symmetrical in all stages of their life cycle.

Before a satisfactory system of classification can be obtained, much more study should be directed to the Anomura. Therefore suggestions for rearrangement, with what evidence is at present available, are quite preliminary, and no real changes can be legitimately made until the life histories of a larger proportion of the members of the tribe are known. However, concerning the genera investigated here, it would seem that the evidence justifies the contention that *Upogebia* and *Paguristes* should not be separated by so great a division as is indicated by their position in separate superfamilies while *Pagurus* and *Paguristes* are included in the same family and separated only into different subfamilies. The facts confirm the position of *Orthopagurus*



1. *Paguristes turgidus* (*Stimpson*), *female*. 2. *Paguristes turgidus* (*Stimpson*), *male*. 3. *Pagurus beringanus* (*Benedict*), *female and male*. 4. *Orthopagurus schmitti* (*Stevens*), *in worm tube, male and female*.

and *Pagurus* within the same subfamily. It is hoped that further study of the life histories of decapod Crustacea will result in a better understanding of the genealogy of the components of the group and in a more natural classification.

Acknowledgments

Facilities for the collection of the adult Anomura and the rearing of their larvae were provided by the Biological Board of Canada, at the Pacific Biological Station, Departure Bay, B.C., from 1929 to 1934. During the winter of 1934–35, financial assistance in the form of a Special Open Fellowship of the University of Toronto, was received, and the work carried on in the Department of Biology at the University of Toronto.

The writer wishes to express her appreciation of the assistance given her by Dr. E. M. Walker in his supervision of the work at the University of Toronto. She is also indebted to Dr. W. A. Clemens for his interest and suggestions during the progress of the experimental work, and would like to thank the members of the staff of the Pacific Biological Station for their helpful co-operation at all times.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 15, SEC. D. NOVEMBER, 1937 NUMBER 11

THE MALE GENITALIA IN THE HYMENOPTERA (INSECTA), ESPECIALLY THE FAMILY ICHNEUMONIDAE[1]

I. COMPARATIVE MORPHOLOGY

BY OSWALD PECK[2]

Abstract

The trend of specialization in the male genitalia and the adjacent sclerites is traced within the order Hymenoptera (Insecta) and the morphological problems of these parts discussed. In the Ichneumonidae, these sclerites appear to have little or no tribal or subfamily characteristics of practical value to the taxonomist, such differentiation being masked extensively by specific variation. Thirty-four tribes and ninety-six species were studied. One hundred and fifty-eight figures are shown.

Introduction

In the family Ichneumonidae, the determination of species is often extremely difficult. The keys to the family are based partially upon the characters of the female and cannot always be relied upon for the identification of the males.

Furthermore, it is not at all certain that the present systematic division of the family is a natural one, the situation being summarized by Cushman and Rohwer (32), who expressed the belief that: "The family Ichneumonidae is a group composed of elements showing remarkable differences but at the same time extreme homogeneity. So true is the latter that the grouping into five universally recognized subfamilies leaves the placing of a species in its proper subfamily almost entirely to the imagination or experience of the worker. On the other hand, the strict interpretation of such characters as these keys offer frequently leads even the experienced taxonomist to entirely misplace an insect; and disagreement among workers as to the allegiance of certain genera is very frequent."

This view was supported by Viereck (135) who keyed out the ichneumonid species without defining either the traditional subfamilies or the tribes. The difficulty in defining the higher groups is shown also by Schmiedeknecht (116), who recognized 13 tribes in the Pimplinae, yet keyed out the genera in the family without direct reference to the tribal grouping.

[1] *Manuscript received October 26, 1937.*
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Under these circumstances, it seemed possible that a thorough study of the male genitalia might help to solve these difficulties.

This viewpoint was supported by the presence of tribal characteristics in the ovipositor of the Pimplinae (32), as well as by the constant elongation of the male claspers peculiar to the *Mesochorini*. Furthermore, while specific characters had been shown in the male genitalia of many other hymenopterous groups, this, in itself, did not preclude the presence of tribal ones as well.

In their structural characters, the members of the family Ichneumonidae are regarded as being intermediate between the Chalastogastra (sawflies and horntails) and the aculeate Hymenoptera (ants, bees and wasps). A study of the comparative morphology of the male genitalia in these forms should thus be of value since it may (1) allow us to recognize and define the different stages of morphological specialization existing within the Order, (2) provide data which will assist in making a more rational arrangement of the larger groups within the Order and the families, and (3) aid in the establishment of a consistent, morphologically sound system of nomenclature within the Order.

The term *genitalia* is usually applied to the organs of copulation, which are morphologically external, although it is sometimes taken as including the internal reproductive organs. Studies of the latter are already available in the Ichneumonidae (13–16, 20, 79) and will not be considered here. However, as the specialization of the hymenopterous genitalia in the male usually involves modifications in the remainder of the genital segment, and these, in turn, affect the tenth and eleventh abdominal segments, the study of the genital region was extended to include these segments also, when necessary.

Comparative Morphology of the Male Genitalia

A. The Male Genitalia in the Hymenoptera

1. *Introduction and Definitions*

An extensive but superficial study of the male genitalia of the Hymenoptera was made by Leon Dufour (34). Supplementary efforts by later workers produced morphological chaos by their failure to give the same names to the same parts. Boulangé (17), by the publication of tables indicating the terms used by the various workers, introduced a semblance of order; however, this author, like Richards (96), preferred to use noncommittal terms, having no precise morphological significance outside of the order, this procedure apparently being based upon the belief, expressed by Richards, that "it is not possible in my opinion to homologise with certainty the parts of the hymenopterous genitalia with those of less specialized orders".

Snodgrass asserted (122, 123) that the parts of the male genitalia in the endopterygote insects can be identified by their musculature and articulatory relationships, although later (124) he described the genitalia "regardless of what may be the morphological relations of the latter". It is certain that in the initial stages of morphological work a non-significant or merely anatomical system of nomenclature is a convenience and, indeed, a necessity; nevertheless,

the establishment of a uniform system of nomenclature, based upon morphological homologies, throughout the whole range of the group studied, is, as all agree, the ultimate objective of morphological investigation.

It seems evident that, if homologization is possible between the ichneumonid genitalia and those of other hymenopterous and endopterygote groups, it must be based upon the primitive sub-order Chalastogastra, its parasitoidal family, the Oryssidae, perhaps being intermediate. Boulangé (17), studied the genitalia and their musculature in the Chalastogastra, in *Vespa* and in *Bombus*. He arranged the various structures in series, the members of each group clearly corresponding in position to one another. However, using myology as the criterion, he regarded the inner clasper of *Bombus*, but not of *Vespa*, as homologous with the volsella (or inner clasper) of the Chalastogastra, even though some similarity in form and function existed in *Vespa*.

As will be shown later in this paper, the parts of the male genitalia iu the Ichneumonidae are clearly homologous with those in the Chalastogastra and therefore, at least, with those in the Apoidea also.

The male genitalia of the parasitoidal Hymenoptera, so far, have received very little attention, though the genitalia of some chalcids were described by Embleton (36), Imms (56), Grandi (44–46), James (57) and Hanna (51): the development of these appendages in the braconid *Doryctes* was observed by Seurat (118) and their structure in a few species of ichneumonids and braconids was studied by other workers (8, 21, 26, 43, 82, 109).

An effort has therefore been made in the present paper to identify the parts of the male genitalia of the ichneumonids with those of the Chalastogastra and with the more generalized forms of insects. Until this is accomplished, it is obviously impossible to place the terminology upon a sound basis.

As the hymenopterous genitalia have a series of sclerites peculiar to this order, a system of names must be selected for these parts, the choice being according to the claims of priority or custom, preferably the former.

The nomenclatorial systems of the various workers were tabulated by Boulangé (17). The earliest of these were proposed by Audouin (6), Hartig (52), Newport (75), Dufour (34) and Schenck (113). Unfortunately, these workers did not confine themselves to the use of similar terms for parts common to the main groups. The application of the law of priority would result in creating a legal monstrosity derived from several systems and thereby losing most of its value; furthermore Audouin's term *spatha* would have to be applied in a sense that he alone recognized, although the term is widely used for the dorsal portion of the intromittent organ. Under these circumstances practicability is the logical criterion.

The next system in order of priority is that of Thomson (127) and there seems to be little to choose between it and that of Hartig. The latter system was sponsored by Rohwer (101) but otherwise was ignored. The former is followed by Mickel (64, 65), Richards (94-96), Ross (108) and Ries (97) who

are some of the chief workers in this field at the present time. Thomson's terms were more widely used than those of any other author and are applicable throughout the order, except, perhaps, with regard to the inner clasper of vespids. They were drawn from the supposed homology or analogy between the gnathal and genital appendages and, to differentiate between them, Crampton (22) suggested that the prefix "gono-" be used for the latter series. To be consistent, it is necessary also to change the term squama to *gonosquama* to prevent confusion with the dipterous squama.

However, Thomson's terms were originally applied to bombids, in which the outer clasper is divided transversely into gonostipes and gonosquama. In the ichneumonids, these are usually fused into a compound structure for which there is no entirely satisfactory term, as the terms gonopod, coxopodite, stylus, harpe and harpago are all morphologically incorrect, while the term stipes-squama of Richards (96) is clumsy, particularly if the prefix gono- is added. The term *forceps* was chosen in 1841 by Dufour (34) but the same term was previously applied to the dermapterous cerci by Burmeister (18). As the word then is not applicable to the outer claspers in the Hymenoptera, the writer proposes the term *gonoforceps* for the latter structure when the gonostipes and gonosquama are fused together.

Since the word *sagitta* was first used for the penis valve in *Bombus* (127), and since this structure is homologous with the paramere, the term should be sunk as a synonym. However, recent writers have used the term for various sclerites, especially for the articulatory sclerite at the apex of the inner clasper; Thomson named this part the lacinia so that, for the sake of consistency, we should now term it the *gonolacinia*.

Other morphological terms used in this paper conform to the usage of Thomson (127), Boulangé (17) and Snodgrass (122-124), excepting the terms aedeagus and paramere, which agree with some concepts of Snodgrass (122, 123) but not his latest (124). Many of the terms, however, are explained in the text. The hypandrium, outer claspers, inner claspers and harpago are considered as denoting function rather than morphological entities.

2. *The Genital and Postgenital Terga*

Among all endopterygote insects, including the Hymenoptera, Trichoptera, Mecoptera, Lepidoptera, Diptera and Coleoptera, the male genital segment is invariably the ninth abdominal. Behind this segment lies the gonopore or genital opening, in the intersegmental membrane and between the two appendages known as the *gonopods* (122, pp. 17, 18).

Among the Hymenoptera, it is but seldom that the tergum of the genital segment is not radically modified. This tergum may undergo a sequence of changes, the various steps, in order, being: (i) invagination, (ii) division into a pair of lateral *genital tergites* (Tg. IX, Figs. 16, 17, 21), (iii) fusion of these genital tergites with the tergum of the tenth segment to form a *syntergum* (Fig. 20) or, alternatively, fusion of the genital tergites with the tergites of

the tenth segment, forming a pair of *syntergites* (Sntt., Figs. 5, 6, 22), and (iv) a reduction in size and functional importance of the syntergites, accompanied often by the loss of the pygopods (Figs. 18, 19).

When the tenth tergum is fused with the ninth, the former may be distinguished by (i) the intimate attachment of the rectum by muscles to the posterior portion of the syntergum, (ii) the points of attachments of the inter- and intra-segmental muscles in comparison with those of the pregenital segments and (iii) the ventro-lateral proximity of the *pygopods* (commonly termed cerci), when these appendages are present. The genital tergites in most ichneumonids have a distinct antecosta; this is absent in the tenth tergum.

In the Chalastogastra, the most primitive group of Hymenoptera in existence, the ninth tergum is usually divided into two lateral tergites. This is true of most siricids (22), although in *Sirex juvencus* L. these tergites are joined by a narrow, sclerotized bridge (17). It is true also of *Xiphidria mellipes* Say (22) and of some species of *Cephus* (17). The incomplete and complete separations of the tergites are shown in *Cephus cinctus* Nort. and *Pteronidea ribesii* Scop. respectively (Tg. IX, Figs. 15, 16).

In contrast to the great majority of the Chalastogastra, the parasitoidal oryssids have the male genitalia completely invaginated (38). In *Oryssus sayi* Westw., as probably in the other members of this sub-order, this invagination has been accompanied by the ninth and tenth terga being reduced in size and in sclerotization, the ninth tergum being divided also into lateral tergites. In this reduction in size, the Oryssidae are more specialized than either the Chalastogastra or the Ichneumonidae. Rohwer (98) noted that cerci (*i.e.*, pygopods) were absent in the Oryssidae; however, in *O. sayi* they are merely concealed by the invagination.

The ichneumonid genital tergum is always divided medianly and may form (i) a pair of tergites (Tg. IX), as in *Pimpla coelebs* Walsh (Fig. 21); this is rare within the family; (ii) a syntergum (Snt), as in *Exeristes roborator* Fabr. (Fig. 20); or (iii) a pair of syntergites, as in *Megarhyssa lunator* L. (Fig. 6) and *Banchus falcatorius* Fabr. (Fig. 22, Sntt.). Intermediate forms also occur.

The chalcids appear to possess a syntergum and a rudimentary eleventh tergum, the syntergum being in many cases identifiable by the possession of pygopods (44-46, 51, 57). It must however be noted that in the figures of these authors the pygopods show that their "ninth tergum" is a syntergum and that Grandi (44-46) incorrectly identified the gonocardo as the tenth segment. The primitive chalcid, *Brachymeria intermedia* Nees, is primitive in this respect also, for the ninth and tenth terga are separate, although the former is medianly divided (Fig. 17).

In the aculeate Hymenoptera, the ninth and tenth terga appear to be always fused (96) and this fusion is usually accompanied by extensive invagination and the loss of the pygopods. In the Chrysididae, only four to six

abdominal terga are visible externally (19). Although both Wheeler (137) and Donisthorpe (33) hold that there are ten distinct abdominal segments in the Formicidae, yet the ant *Lasius niger* L. has the sclerotic area of the syntergites almost entirely reduced, although their identity is shown by the prominent pygopods (Fig. 18, Pyg.). Syntergites (Sntt.) are present also in *Vespa maculata* L. (Fig. 19), *V. germanica* F. (17), *Bombus terrestris* L. (17), *Colletes cunicularius* L. (72), *Andrena wilkella* (Kby.) Ill. (Fig. 14), *Halictus lerouxii* Latr. (Fig. 13) and in *Apis mellifica* L. (120). This agrees with the contention of Richards (96) that, in the aculeate Hymenoptera, the ninth tergum is apparently always reduced to a pair of small syntergites.

While it is evident that the ninth tergum, when fused to the sclerite or sclerites posterior to it, is indubitably in direct contact with the tenth tergum, yet the morphological significance of the latter sclerite is not entirely plain. As noted above, the pygopods are borne upon the tenth segment. According to Boulangé (17, p. 218), they are appendages of the tergum, although Middleton (66) has rightly claimed that in *Pteronidea ribesii* they lie ventrally to this sclerite. However, the series of chalastogastrous and ichneumonid adults examined during this study suggest that the pygopods lie immediately postero-ventrally to the tergum, arising in the intersegmental membrane. Snodgrass (122, 124) holds the view that they are not cerci (*i.e.*, not appendages of the eleventh segment) but may be homologous with the socii of the Lepidoptera. Whatever their morphological significance may be, these appendages serve as admirable landmarks of the postgenital segments.

The eleventh tergum varies considerably, both in the degree of sclerotization and in its relationship to the tenth tergum. Berlese (11) claimed that the tenth and eleventh terga were fused together in *Cimbex americanus* L. and separate in *C. axillaris* Pz. This inter-tergal fusion was believed by Crampton to probably form the chalastogastrous epiproct (22). Boulangé (17) found in *Xeris spectrum* L. (Siricidae) and *Cephus pygmaeus* L. (Cephidae) evidence of fusion through the persistence, in an attenuated state, of the musculature of the two postgenital terga. The same author believed that a similar fusion occurred in *Bombus* and *Vespa*.

However, Snodgrass (122, p. 97; 124, pp. 253, 605) considered that the postgenital dorsal sclerite in the Hymenoptera is that of the tenth alone. This is supported by the existence of a broad membranous area behind the last sclerite in many chalastogastrous and ichneumonid species. In the sawflies *Pteronidea ribesii* and *Dolerus unicolor* Beauvois this sclerite bears strong setae and setal alveoli respectively, the setae being similar to those of the tenth tergum; in these cases, the setal remains surely must be landmarks of a primarily sclerotic area, which can only be the eleventh tergum. This identification is also supported by the invariable occurrence of the ichneumonid pygopods in the membrane immediately behind the last functional tergum, suggesting that the eleventh tergum is invariably either fused to the tenth or else de-sclerotized, at least in the ichneumonids.

The further exploration of this problem is outside the scope of this paper, but the morphological value of the pygopods as landmarks makes some reference to it essential, in order to show the significance of the post-genital terga and therefore of the syntergum and the syntergites.

3. *The Genital and Postgenital Sterna*

Iu the Hymenoptera the ninth sternum may be specialized by (i) invagination, the ninth sternum lying dorsally to the eighth, (ii) reduction in sclerotization and (iii) fusion with the eighth sternum. The hymenopterous *hypandrium* is usually formed of the ninth sternum alone, but in at least some of the Vespidae, the eighth sternum is fused with the ninth. The composite character of the hypandrium in the latter case is usually indicated by the presence of (i) the median *spiculum*; (ii) the *antecostae* of the two sterna and (iii) the inter-segmental and intra-segmental muscles.

The ninth sternum is well developed in the lower Hymenoptera, such as the Chalastogastra (17), the Idiogastra and the ichneumonids, as well as chalcids (44-46, 51, 57). The aculeate hypandrium is reduced in size and lies dorsally to the eighth (17, 70-72, 110-112). Atwood (5) regarded the eighth and ninth sterna of *Halictus* and *Andrena* as formed by a secondary division of the eighth, the gonocardo being the "base of the ninth ventral segment". In *Halictus lerouxii* and *Andrena wilkella*, a strong muscle (Boulangé's *muscle en sangle*) extends from the anterior part of the syntergite (*i.e.*, from the genital tergite) to the ninth sternum and short inter-sternal muscles exist between the eighth and ninth sterna. In *Andrena wilkella* the acrosternites (Ast.) and antecostae (Ac.) of the two sterna are plain (Fig. 14). Furthermore, in this species, the spiculum (Sp.) has been bent posteriorly through invagination, forming an internal median ridge upon the ninth sternum. In Atwood's figures, the antecosta and spiculum of the ninth sternum are plainly shown as darkly stippled areas. In *Apis mellifica* the ninth sternum is "a well-developed semicircular band, forming the ventral and ventro-lateral parts of the ninth segment. It bears on each side, two conspicuous lobes" (Snodgrass (120)). These lobes are the claspers.

Among the wasps, specialization has been carried further by the fusion of the eighth and ninth sterna (17, 58, 132, 138). The vespid hypandrium has been incorrectly interpreted as the eighth sternum and the gonocardo as the ninth by Balfour-Brown (7). The hypandrium of *Vespa* is composed of two sterna (17), while the two antecostae, spiculum, and segmental muscles are also prominent (Figs. 36, 37).

In contrast with the development and subsequent degeneration of the ninth sternum, the tenth and eleventh are always retrograde structures, usually being indefinitely demarcated and semi-membranous. This is plainly due to their early invagination between the terga and the gonopods.

In some ichneumonids, notably *Pimpla instigator* Fabr. and *Megarhyssa lunator* Fabr., the rigidity of the post-genital sterna is still well preserved. All ichneumonids have the ventral margin of the anus and the adjoining

rectum supported by a sclerotic area; the latter is usually divided medianly and varies in shape from hyperbolic to V-shaped and quadrate. In *Ephialtes tuberculatus* Auct., *nec* Fourcr., these sclerites bear strong setae with large alveoli, suggesting strongly that these anal structures are not secondarily developed but are remnants of the eleventh sternum, particularly as they lie ventrally to the membranous area that seems to be the eleventh tergum.

In the chalcid, *Brachymeria intermedia*, the post-genital sterna appear to have fused, forming a long rod that joins the lower lip of the anus to the base of the genitalia (Fig. 38); its fusion is not clear and no modification of this kind has been described in other chalcids by Grandi (44-46), James (57) or Hanna (51).

As the anal sterna are fragile and minute, as well as being only indirectly concerned with genitalia, they have not been included in this study; however, they appear to have acquired their present form early in the evolution of the Hymenoptera and may possess group characteristics of interest.

4. *The Genital Appendages*

Since the basal, annular gonocardo is derived from the gonopods, it is an integral part of the male genitalia in the Hymenoptera, although in recent years it has been misinterpreted as the ninth segment by Grandi (44-46), Balfour-Browne (7), Atwood (5) and Abbott (2).

(a) The Typical Structure. The male genitalia differ considerably in the various families of the Hymenoptera, yet all exhibit the same fundamental structure, as shown by Boulangé (17) in the Chalastogastra (Figs. 9-11). Except in the honey bee, four main parts are distinguishable in the adult, these being (i) the basal annular *gonocardo* (Gc.), which bears latero-posteriorly (ii) a pair of hollow, hemi-ellipsoidal outer claspers or *gonoforcipes* (Gf.), each of which supports by its antero-ventral margin (iii) the inner clasper or *volsella*. The gonoforcipes and volsellae support, by means of muscles and membrane, a median intromittent organ, later shown to be (iv) an *aedeagus* (Aed.).

The gonocardo is attached to the intersegmental membrane lying posteriorly to the ninth segment; it sometimes bears a medio-ventral apophysis, the *gonocondyle* (17). Dorsally to the gonocondyle or gonocondylar area lies the tip of the *gonostipital arm*, an anterior elongation of the *gonostipes* or basal portion of the gonoforceps. The apical part of the gonoforceps is frequently differentiated into a distinct appendage, the gonosquama, which may be articulated. Gonosquamae are present in most Chalastogastra (22), in a few ichneumonids and vespids, in bombids and in other aculeate Hymenoptera; in the ichneumonids they are not articulated.

The gonostipes bears the volsella, which, among the lower families, normally lies in the vertical plane. The volsella often assumes bizarre shapes. It frequently bears an apical articulatory sclerite, designated in this paper the *gonolacinia*, although termed the *pièce en trébuchet* by Boulangé (17), the *middle clasper* by Peacock (80) and the *squama* by Salt (109) and Glover (43). The word squama was originally applied by Thomson (127) to the apical portion of the outer clasper in *Bombus*.

The aedeagus has no close articulation with the remainder of the genital sclerites. In its unspecialized form this organ consists of a pair of lateral *parameres*, sometimes termed the *sagittae*, joined both dorsally and ventrally by membrane which may become secondarily sclerotized. The dorsal portion of this membrane may be distinguished as a heavily sclerotized area and then is termed a *spatha*. Each of its antero-lateral corners forms a lateral apophysis, Boulangé's *ergot*, which forms a fulcrum about which the aedeagus can pivot in the sagittal plane; the ergot is usually strengthened by accessory *spathal rods*. Anteriorly the parameres extend far into the body cavity. The aedeagus may assume remarkable modifications in form and size, as in *Bombus*. It is usually large in the chalcids (34, 36, 44-46, 51, 56, 57), dwarfing the small outer and inner claspers. In the hive bee also the aedeagus is abnormally large but, being eversible, it is mainly membranous (120).

(b) Ontogenetic Development. In all Hymenoptera the histoblasts of the male gonopods are situated in the twelfth larval segment behind the head (*i.e.*, the ninth abdominal segment of the adult). This has been demonstrated in the Chalastogastra (17), Proctotrupidae (35), Ichneumonidae (119, 126), Braconidae (42, 76, 131), in the Vespidae, Bombidae and Apidae (138) and in other groups.

A study of the ontogenetic development in *Sirex* (17), in the braconid *Doryctes* (118) and in *Vespa* and *Bombus* (138) shows that each histoblast develops into a primary papilla that divides longitudinally into the outer and inner claspers. A transverse basal sclerite is separated from the base of each primary papilla, fusing with its homologue from the other gonopod to form the annular gonocardo.

According to Zander (138-140) the parameral papilla in Lepidoptera, Trichoptera and Hymenoptera is formed from the base of each primary papilla, soon to fuse with its fellow and with the ejaculatory duct to form an aedeagus. This mode of the formation of the parameres was generally accepted as true until recently, when Mehta (62) claimed that the parameres in Lepidoptera develop earlier than the lobes of the gonopod and independently of the primary papilla. Moreover, Mehta was able to support his ideas by citing evidence from the Hymenoptera (63) and from the other main orders in the Endopterygota. The significance of this is discussed in the next section.

Among the Hymenoptera the formation of the genitalia is always along somewhat similar lines, but variations occur, both in the number of adult appendages and in the order of their differentiation. Unfortunately, most workers upon this aspect studied the genitalia of the honey bee, in which these structures are aberrant: only two pairs of secondary papillae have been found in this insect so either the parameres are absent (120, 138) or else the outer and inner claspers remain undifferentiated (17, 63).

Of greater interest is the disagreement between the developmental and myological evidence. The chronological development in *Sirex*, *Vespa* and *Bombus* is shown diagramatically (Fig. 1); unfortunately, the description of the braconid *Doryctes* by Seurat (118) is inadequate for this purpose. The

differentiation of the parameres (Pr.) occurs later than the separation of the individual outer and inner claspers (O.C. and I.C.), both in *Sirex* (17) and *Vespa* (138), although their volsellar muscles are not homologous (17). Yet the reverse is true of *Bombus*, for the parameres are differentiated before the appearance of the two pairs of claspers (138), although the genital muscles are homologous with those of *Sirex* (17).

The discrepancies between these data suggest that the ontogenetic evidence should be interpreted cautiously but suggest the independence of the parameres from the gonopod and thereby provide support for the contention of Mehta (62) that the claspers but *not* the parameres are gonopodal in origin.

Before our available ontogenetic data can be of value in studies of the hymenopterous genitalia, they must be adequately verified and supplemented. At present they appear to be of little use in comparing the genitalia of adults, unless supported by other evidence.

(c) The Morphological Significance. According to Snodgrass (122, 123), the typical male gonopod of an adult insect consists fundamentally of a basal *coxopodite* and an apical stylus or *telopodite*, the coxopodite bearing an unsegmented *paramere* on its inner margin. The coxopodite may be free, fused to its fellow, or joined to other parts of the genital segment. The parameres of the higher insects are fused to the terminal portion of the ejaculatory duct, forming the median intromittent organ, while the stylus is represented by the clasper; each of the latter may be divided into a pair of claspers also (122, p. 192). Accessory structures may be present but almost invariably they have no muscles and are therefore distinguishable from the paramere and stylus, which are attached by muscles to the coxopodite.

It must be remembered that the origin and position of appendages are not the sole guides to their homologies, for the direction of articulation of limbs is an unusually stable landmark (122). Since the gonopods are believed to be homodynamous with the thoracic limbs, a comparison of the genital claspers with the primitive thoracic limb may be of value in determining the homologies of the genitalia.

The primitive thoracic limb consists of the basal coxopodite (Cxp.) and the distal telopodite (Tlp.) with muscles extending from the base of the coxopodite to the base of the telopodite, as in Fig. 2 (121, 124). The coxopodite moves in a horizontal plane upon the body, the secondarily differentiated coxa moving in the same plane also, while the telopodite articulates vertically upon the coxopodite (121, 124), as shown in Fig. 4. Since both the primary papillae of the thoracic limbs and the genital histoblasts are formed upon the ventral surface of the body, the initial inherent movement of the undifferentiated coxopodite is sagittal. The subsequent development of the insect causes the thoracic papillae to migrate laterally, while the genital appendages move posteriorly; the coxopodite of the latter therefore secondarily articulates in a vertical plane, while the telopodite moves horizontally (121). The significance of this is shown later.

The term *paramere* was proposed by Verhoeff (133) for a paired appendage lying laterally at the base of the coleopterous penis. Since this time, this term has been applied loosely, especially when the parameres are united as an aedeagus. The location of the coleopterous parameres upon the penis shows that these structures are phallic, not gonopodal, and with this Snodgrass (124) concurs. Yet the parameres of the endopterygote orders are widely believed to be gonopodal in origin, as stated by Snodgrass (122, 123). Snodgrass' conclusions upon the origin of the parameres may also have to be modified if the above-mentioned ontogenetic findings of Mehta (62), prove to be valid. Substantiated by the data of workers in other orders, Mehta's work indicated the probability that the basic data, as outlined by Snodgrass (122, 123), are partially incorrect, the parameres never being gonopodal. Should this be so, Mehta has clarified considerably the current concepts, not only upon parameres, but also upon the fundamental structure of the male genitalia in the Endopterygota, a group in which a common basic form is to be expected.

Recently Snodgrass (124) has reviewed the male genitalia of pterygote insects and his conclusions are quite at variance with his previous ones (122, 123). The term paramere is accepted in the coleopterous sense but in this work he has adopted a terminology "that can be applied consistently to the major structural elements *regardless of what may be the morphological relations of the latter*" (the italics are mine). The genital structures are divided into two classes, phallic and periphallic. The phallic organs are "immediately concerned with the function of coition; they include the phallus and various accessory or supporting structures associated with the latter. The periphallic organs are movable or immovable lobes or processes that have for the most part a grasping or clasping role in the function of copulation" (p. 586). The phallic organs, including the parameres, are evolved from the conjunctival membrane posterior to the ninth sternum or in the genital chamber anterior to the membrane. The "periphallic structures .... arise peripherally, generally from the annulus of the ninth abdominal segment but also from the other segments often closely associated with the latter in the genital complex" (p. 591). They may include a pair of lateral movable claspers and various immovable lobes or processes arising from the tergum or sternum; the movable claspers or *harpagones*. To me, these concepts seem inadequate for the positive identification of some of the genital appendages.

Applying these concepts to the Hymenoptera, Snodgrass suggests that their genitalia are entirely phallic, the gonocardo and gonostipites being formed from the phallobase, while the median appendage is an aedeagus; the gonosquamae "may be termed parameres since they are at least analogous with the parameres of Coleoptera", even though he states that the apices of the parapenes are "structures of the same nature" as the gonosquamae. How little is realized of the morphology of these hymenopterous structures is shown by these suggestions that the parameres may be either the gonosquamae or parapenes, or, if the word aedeagus is to be construed rigidly, even a part of this median organ also.

Snodgrass' conclusions are not entirely consistent with his assumptions, the claspers in the Hymenoptera being considered as radically different from those of allied orders. The claspers in the Lepidoptera arise from the genital cavity (62, 140), as do those of the Hymenoptera (63, 138); yet the former are termed periphallic and the latter phallic, even though their functions are similar. Furthermore, the derivations of the main genital parts in the Lepidoptera, Trichoptera and Hymenoptera are at least similar enough to permit their homologization by Zander (138-140). Even the muscles at the base of the clasper in *Panorpa germanica* L. suggest the affinity of this structure with that of the Hymenoptera (17); yet the mecopterous appendages are termed periphallic. Because of these data, the hymenopterous clasper seems to be homologous with those of allied orders.

The parameres and other genital appendages have been identified also by Pruthi (85). This author considered the parameres to be derived from the median papillae and distinct from the gonopods, which are in the same general plane as the ninth sternum, lying under the penis and parameres. Thus Pruthi agrees with Mehta (62) that the parameres are attached to the base of the penis (it should be noted that Pruthi has interpreted the penis as the aedeagus).

From the ontogenetic data it is plain that the hymenopterous gonopod is basally fused to its fellow, forming the gonocardo (142). This fusion is indicated in the adult by a medio-ventral or medio-dorsal suture or else a corresponding internal ridge; the ventral fusion is shown in many species of ichneumonids, while the dorsal fusion is plainly shown in *Nototrachys foliator* Fabr. and *Trogus lutorius* Fabr. The gonocardo therefore must represent either the two coxopodites or else their bases.

As the median intromittent organ is derived from the fusion of the ejaculatory duct with paired rudiments, this composite organ is evidently an aedeagus. The Chalastogastra, a primitive group in the order, has a primitive aedeagus also, for it often consists almost entirely of the duct, of the two elongate sagittae and of connective membrane; it seems evident that the parameres are represented by the sagittae, especially since the latter are found throughout the members of the order (except possibly *Apis mellifica*) and form the inflexible lateral parts of the compound organ.

The outer and inner claspers are provided with well developed muscles, so that according to Snodgrass (122) neither can be purely accessory structures. Crampton (23, 24) suggested that the gonosquama is the stylus and Richards (96) supported him by indicating that the gonostipes and gonosquama probably form the apical portion of the coxopodite with the stylus. The latter author suggested also that the volsella is a two-segmented paramere and the median organ an aedeagus; his basic definitions evidently differ from those of Snodgrass (122, 123). Becker (8) concluded that the volsella of the Tenthredinidae and Ichneumonidae represents the trochanter and femur, and the bombid volsella the femur. The gonosquama then presumably would

be an epipodite. As Becker appears to have compared the hymenopterous genitalia directly with those of the Apterygota, his failure to study intermediate forms seems to invalidate his conclusions entirely.

A homology between the volsella and the telopodite is suggested by the constant presence of the articulating gonolacinia in the lower families of the Hymenoptera. Moreover, the volsella in the Chalastogastra and Ichneumonidae has a constant, diagonal, internal thickening, the *volsellar strut* (V. Str.) (Figs. 9, 117–127). This suggests the remnant of the arthrodial thickening in a flattened limb joint, the adjacent limbs being joined by Boulangé's muscle Q (Fig. 8). However, this strut appears to be secondary, for its function is to prevent buckling of the volsella during the contraction of Boulangé's muscle Q. Furthermore, there are no condyles in the gonostipito-volsellar joint, although they should be present in an articulatory limb that is derived from a primitive leg.

The lack of homology between the volsella and the telopodite is further shown by the base of the volsella being almost flat in the primitive Hymenoptera, so that, if this homology were correct, the telopodite must have changed basally from the normal tubular form to one that has been either very strongly compressed or else split along the main axis of the limb, spread outwards and flattened.

The first method of evolution is impossible, for there is no sign of fusion between the opposite sides of the limb (except possibly the volsellar strut), the basivolsella being thin and almost diaphanous. Furthermore, the intrinsic muscles of the tubular limb must have been gradually replaced by (or developed into) an efficient and complex extrinsic system. It is absurd to postulate the occurrence of both of these radical changes, each of which is highly improbable.

The second method of formation seems to be equally impossible. The longitudinal fission and unfolding of the basal part of the telopodite, accompanied by extensive internal changes, could only have evolved slowly, yet intermediate forms appear to be absent. Furthermore, this change from the tubular form to that of a plate would weaken the telopodite, probably at the very time that it was being adapted as a clasper, a structure that is essentially strong. This theory can be accepted only if accompanied by further proof, and in the absence of a more logical explanation.

The absence of homology between the telopodite and the volsella is shown by the origin of the latter. This structure is formed from the lateral papilla by an apical or medio-apical fission, the volsellar region being the smaller (Fig. 1). This suggests that the volsella is not the entire telopodite, although possibly an endite.

The fundamental difference between the telopodite and the volsella is shown also by musculature. The base of the paramere is joined to the base of the gonolacinia by Boulangé's muscle N in both the Chalastogastra (Fig. 8) and the Ichneumonidae (Fig. 7). Even if it is conceded that the paramere is gonopodal in origin, one cannot recognize the existence of a primary muscle

that extends between a basal appendage of the coxopodite and the apical telopodite. Either this muscle is secondary (and secondary muscles are usually short) or else the volsella is not part of the telopodite.

The morphological relationship between the gonopod and the volsella is emphasized by the detailed adult structure of the latter.

The separation of the volsella and gonostipes is anteriorly incomplete in some siricids, as in a species of *Sirex* (39, Fig. 15) and in both *Sirex juvencus* and *Xeris spectrum* (17, p. 228); the same phenomenon occurs in widely separated species of ichneumonids (Fig. 97), and is discussed later. While this is indicative of a similar morphological relationship between the inner claspers of these groups, yet, coupled with the primitive nature of the family Siricidae, it further suggests that the volsella originated from the gonoforceps.

The view that these two structures are primitively one is supported by the appearance of the upright ichneumonid basivolsella, in which (i) setae and alveoli are present facing the aedeagus, (ii) the muscles lie extrinsically and laterally and (iii) the curvature is towards the aedeagus. Together, these factors strongly suggest the rounded shape and musculature of the mesal portion of the gonopod. The setae are evidently primary, because they are small in comparison with the alveoli; furthermore, the setae and alveoli only occur postero-ventrally, as in *Ichneumon grotei* Cress. (Fig. 121), this being a position favored by the basivolsellar curvature for the survival of setae from the attritional effects of invagination, yet an unsuitable place for the development of tactile organs for use during coition. The curvature of the basivolsella is in itself insignificant, for the shape may possibly be due to the tension exerted by Boulangé's muscle Q (Fig. 8), although this muscle probably is only used during coition. Individually these three factors are not of much weight, yet, when correlated with each other and with the ontogenetic development of the volsella, they indicate that the volsella and gonoforceps in the Ichneumonidae are primitively one structure.

These facts, incidentally, serve also to show the falsity of the idea that the volsella may have originated directly from membrane, instead of from sclerotic structures; the form and musculature of the volsella are far too specialized to be associated with such an extensive change, one that is already so complete that there are no intermediate forms, even in the most primitive of the Hymenoptera.

Since the volsella and gonostipes are secondarily divided from each other, the gonopod consists of either three or four units, these being (i) the lateral half of the gonocardo, (ii) the gonostipes and volsella, (iii) the gonosquama and possibly (iv) the paramere of the aedeagus. Unfortunately, these units do not articulate sufficiently in any one plane to allow recognition of the coxopodite, subcoxa or coxa.

Neither can these parts be identified by their musculature. The longest muscles in the male hymenopterons are Boulangé's muscles I, J, M and N (Figs. 8, 10), all of these being attached to the base of the paramere and to the apex of either the gonostipes or the volsella. Consequently, if the para-

mere is held to be fundamentally separate from the gonopod, these muscles must be attached to the coxopodite and not to the telopodite, the gonostipes and volsella then being derived from the coxopodite. On the other hand, if the paramere is an appendage attached to the base of the gonopod, the gonostipes and volsella must still be derived from the coxopodite, else muscles are extending from the base of one appendage to the base of another. Crampton (24) has cited Snodgrass as considering this derivation to be the case.

However, this conclusion may seem to be invalidated by the gonocardo being constantly the first and only sclerite to be transversely differentiated from the gonopods. On this account, the two coxopodites may be regarded as being modified as the gonocardo. The morphological importance of the latter was emphasized by Pruthi (85), who suggested that it was probably homologous with the structures to be found at the base of the genitalia in the other main pterygote orders.

Assuming that both the gonopod and the thoracic limb are primitively divided into the coxopodite and telopodite, there would seem to be but one way of reconciling the ontogenetic and myological data. It can be put forward that the primary muscles of the gonopod have degenerated, owing to the extensive structural modifications that the gonopod has undergone. As pointed out by Abbott (2), there is no muscle capable of moving the ichneumonid claspers laterally; the genitalia then must be extended sideways by an increase in the blood pressure in this region. Blood pressure has already been noted as a factor in the functioning of the genitalia of the male honey bee (120), although in this insect there are no functional claspers and the aedeagus is mainly membranous. In the ichneumonids, too, the volsella is rotated from the vertical or resting position to the horizontal or copulatory attitude and, as there are no muscles capable of doing so, again an increase in the blood pressure in the neighborhood must be responsible. Should this modification be generally true of the Hymenoptera, then the arrangement of muscles is not a reliable source from which to draw fundamental, morphological conclusions.

The true nature of the hymenopterous gonopod, then, is not as yet definitely established. The median organ is an aedeagus, while the gonocardo *may* represent the fused coxopodites, the remainder then being derived from the telopodite. However, the stipes and volsella are primitively one. Until further evidence is found, it seems impossible to homologize with certainty the hymenopterous genitalia with the primitive limb.

B. The Ichneumonid Male Genitalia

1. *The Structure and Musculature*

The ichneumonid genitalia resemble those of the Chalastogastra, a closely related but less specialized group, in which however the genitalia vary greatly in form. The ichneumonids differ from the Chalastogastra in (i) the frequent formation of a syntergum or of a pair of syntergites, (ii) the almost invariable

absence of the parapenes, (iii) the absence of a distinct gonosquama in most species, (iv) the greater refinement or specialization of the volsella and its greater uniformity of shape and (v) the greater degree of sclerotization in the aedeagus. The ichneumonids are nearer to the parasitic sub-order Idiogastra, as represented by *Oryssus sayi*, although this species has a non-articulated gonosquama and its aedeagus is only weakly sclerotized.

The main homologies between the chalastogastrous genitalia and those of the ichneumonids are quite evident, if shape, position and articulation are sufficient criteria. This, however, does not apply to the inner claspers, and to show this relationship it is necessary to describe in detail the structure of the ichneumonid inner clasper. This appendage is assumed to be a volsella and the proof is shown subsequently.

The volsella of *Neotypus americanus* Cush. is typical of this structure as it occurs in the ichneumonids so that both the external (or mesal) and internal views of this organ in the vertical position have been shown (Figs. 119, 120). It should be noted that all drawings of this appendage show a large basal piece shaded by broken lines; these lines do not represent a completely de-sclerotized plate, but, instead, one that is extremely thin compared with the remainder of the volsella, almost resembling membrane in appearance. This plate is termed the *basivolsella* (Bv.) and is braced by the internal *volsellar strut* (V. Str.) which divides the basivolsella by passing from the antero-ventral margin to the postero-dorsal corner of the basivolsella. The anterior apex of the strut is developed into the *basivolsellar apodeme* (Bv. A), which is curved ventrally, laterally or dorsally. The postero-ventral margin of the basivolsella bears the strongly sclerotized *distivolsella* (Dv.) in which the apical margins are usually incurved and fused, forming a hood. The antero-dorsal margin of the distivolsella is prolonged into the *distivolsellar apodeme* (Dv. A.) to which is attached dorsally the gonolacinia (Gl.), which almost invariably is articulated.

The volsellae in most sawflies bear but little resemblance to the inner claspers of the ichneumonids, except that they have in common two distinct sclerites, the anterior *pièce complémentaire* of Boulangé (17) (which corresponds to the basivolsella and distivolsella) and the dorso-apical *pièce en trébuchet* (which is identified with the gonolacinia).

The position and articulation of the ichneumonid inner clasper is quite similar to that of the Chalastogastra. The same variation in articulation occurs in both, since the siricid volsella moves either feebly or not at all upon the gonostipes, although freely in the tenthredinids (17, p. 228); the former is true of a few species of ichneumonids, although the latter is far more common.

The volsellar strut is a constant feature of the ichneumonid inner clasper and is plainly identical in position with the diagonal nervure of Boulangé (17, pp. 62, 97), as shown in the siricids and in the tenthredinid, *Tenthredella mesomelas* L. (Fig. 117). It occurs also in the tenthredinid, *Dolerus similis*

Nort. (Fig. 118), in which the anterior end is developed into an apophysis, corresponding to the anterior apodeme of the ichneumonids.

The apex of the *pièce complémentaire* in both *T. mesomelas* (Fig. 117) and in *D. similis* (Fig. 118) shows a striking similarity to the ichneumonid distivolsella in having the apical hood and the antero-dorsal apodeme. Furthermore, this apodeme is present in *Oryssus sayi* (Fig. 113*) a member of the intermediate, parasitoidal Idiogastra.

Nevertheless, the diversification of form in the Chalastogastra suggests that there is a possibility that these similarities are due only to convergent evolution and thus are insignificant; this is supported by the view held by Boulangé that the inner clasper of the vespids is not homologous with the volsella of the Chalastogastra. On this account, the muscles of the chalastogastrous and ichneumonid genitalia (and the adjacent sclerites) have been compared.

The pimpline *Megarhyssa lunator* was selected for this work since its great size allowed the use of the binoculars instead of the microtome, the lack of appreciable amounts of melanin permitting the satisfactory transmission of light through the sclerites; the elongation of the abdomen and of the genitalia tends to isolate many of the muscles so that they can easily be recognized, but sometimes it also tends to change their axes. The muscles of *M. lunator* are compared with the morphological type of the Chalastogastra, as established by Boulangé (17), whose system of myological nomenclature is therefore followed (Figs. 7–11). Muscles D, E, F and L are not shown in the illustrations of *M. lunator* (Fig. 7) but their positions are described. A few of the muscles and their possible functions in *M. lunator* have already been briefly described by Abbott (2).

The abdomen of *M. lunator* is both narrow and elongate, the sterna being so strongly invaginated that the ventral margins of the terga touch in places. No muscles appear to join the tergum of one abdominal segment to the sternum of the immediately anterior or posterior segment (Fig. 5); the absence of this type of oblique muscle identifies the lateral parts of the syntergites as the genital tergites (Fig. 6), their fusion to the tenth tergites being incomplete posteriorly. The posterior boundary of the latter segment is indefinite, since the tenthredinid pygopods are borne upon the tenth tergum (124), although the ichneumonid pygopods are invariably separated from the tenth tergum by some membrane, this space being accentuated in *Megarhyssa* so that it resembles the eleventh tergum.

The syntergites are joined to the ninth sternum by a well developed muscle, Boulangé's *muscle en sangle*. The hypandrium is joined to the gonocardo by muscles A, B and C; the first two extend from the spiculum to the gonocondyle and to the antero-lateral margin of the gonocardo respectively, lying closely together, as in *Sirex juvencus*. Muscle C is weak but distinct in *M. lunator*, extending between the latero-posterior portion of the hypandrium

*This figure supports the tentative conclusions held by Crampton (22) in regard to the volsellar structures in the Oryssidae.

and the gonocondyle. The ichneumonid gonocondyle resembles that of Orthandria in being either minute or absent; the development of the gonocondyle is associated with the lateral torsion of the genitalia in the Strophandria (22)*.

The gonocardo is joined to the basal margin of each gonostipes by muscles D, E, F and G; D and E cross each other ventrally, while F and G are dorsal. Muscle D is attached medianly to the gonocondyle and E similarly to the gonostipital arm. As *M. lunator* has long gonostipital arms, D and E lie dorsally to the gonocardo, E being short. Muscle F extends laterally from the antero-median portion of the gonocardo and is rudimentary, for this part of the gonocardo in *M. lunator* is membranous. Muscle G joins the antero-lateral margin of the gonocardo to the antero-median margin of the gonostipes, and its direction has been changed from being almost transverse to nearly longitudinal, owing to the sagittal elongation of the gonocardo; G has therefore assumed a part of the function of F.

Five muscles, H–L, join the gonostipes to the aedeagus. Muscle H is narrowly attached to the base of the paramere and broadly to the base of the gonostipital arm; it is well developed in *Megarhyssa;* its function is that of raising the apex of the aedeagus, which is balanced about the ergots by membrane and muscles. Muscle I, connecting the gonostipital arm to the ergot, is strong in *M. lunator* and serves to retract the aedeagus, sharing this function with the slender muscle K, which extends antero-dorsally from between the ergots to the gonostipes. Muscle J causes the exsertion of the aedeagus and connects the base of the paramere to the postero-dorsal part of the gonostipes; J is long and strong in *Megarhyssa* and in the Tenthredinids, although short in *Sirex*, *Bombus* and *Vespa* (17). The length of J in *M. lunator* may be due to the elongation of the gonostipes but then the same should be true of *Vespa*, unless only the apical part of the vespid gonostipital area has been developed posteriorly. Muscle L extends laterally from the ergot to the gonostipes and is both short and weak in *Megarhyssa*.

In *Megarhyssa* the gonosquama appears to be incompletely fused; muscle T is present, extending from the baso-lateral portion of the gonostipes to the gonosquama; the short gonosquamo-gonostipital muscle U and the intrinsic gonosquamal muscle V were not identified in *Megarhyssa*, perhaps because the gonosquama does not articulate in this species.

The volsella articulates only weakly in the siricids but strongly in the tenthredinids (17) so that the muscles of the ichneumonid volsella should resemble those of the latter group. Muscles O and P unite the volsellae to the gonostipites. Muscle O is absent in the Orthandria and in *M. lunator* but joins the base of the tenthredinid basivolsella to the apico-lateral part of the gonostipes. Muscle P, joining the antero-lateral margin of the gonostipes to the distivolsella, is present in all of these groups; it is well developed in *Megarhyssa* and is attached broadly at the base (this is not shown in Fig. 7).

**In the Strophandria or Tenthredinoidea the male genitalia rotate through 180° on the median axis before eclosion; this does not occur in the Orthandria (17, 22, 108).*

Two muscles, M and N, join the volsella to the aedeagus. The former extends from the apex of the gonolacinia to the extreme base of the paramere and is absent both in the Tenthredinidae and in *M. lunator*, although present in siricids. Muscle N joins the chalastogastrous gonolacinia basally to the base of the paramere. A moderately weak muscle is attached to the base of the gonolacinia in *Megarhyssa* but its basal attachment was not definitely established.

The volsella has three intrinsic muscles, Q, R and S, each attached to the base of the basivolsella and extending respectively to the apex of the distivolsella, the apex of the gonolacinia and the base of the gonolacinia. Muscle Q is present in the siricids and is large in *M. lunator;* in the tenthredinids it appears to be attached to the gonolacinia but Boulangé (17, p. 71) is not definite upon this point. In *M. lunator* the attachment is definitely at the basal apodeme of the distivolsella. Muscle R is absent in *Megarhyssa*, as there are no muscular fibres visible within the gonolacinia, while S seems to be reduced to some weak fibres.

The muscles of *M. lunator* disagree in detail with those of either the Orthandria or Strophandria alone, but when these are considered together, there can be no doubt that the gonocardo, gonostipes, gonosquama, basivolsella, distivolsella, gonolacinia and aedeagus are homologous in the three groups, the basi- and distivolsella together corresponding to Boulangé's *pièce complémentaire.*

2. *Function*

While much has been written upon courtship among the Hymenoptera, including some ichneumonids, only a few inadequate references have been made to the function of the genital parts of the male ichneumonid during coition or, indeed, even to the coital attitude of the male.

As early as 1799, De Geer (41) described the seizure of the female subgenital plate (*i.e.*, the eighth sternum) by the outer claspers of a male ichneumon, while Rohwer (100) noted that the inner claspers and aedeagus in the sawfly *Euura macgillrayi* Roh. were inserted into the genital cavity at the base of the subgenital plate. Neither Boulangé's own work nor his review of the literature adds anything further in regard to the functioning of the inner claspers and aedeagus.

The usual ichneumonid position of copulation seems to be that of *Pimpla instigator*, in which the male is dorsal to the female with his abdomen curved below the tip of the female's and somewhat to one side in order to avoid the ovipositor. In *Megarhyssa lunator* the male lies above the female with the apex of his abdomen below that of the female and the aedeagus is inserted from the anterior direction, since the female gonopore opens anteriorly (1, 2). In *Paniscus*, the male finally assumes a pendent position (130); this may be true of *Angitia fenestralis* Hlmgr. also, for specimens taken *in copula* were only loosely held together after being killed in alcohol and this may be due, not to chemical reactions upon the muscles, but to the adults having died

before the pendent attitude was reached. This variation in copulatory position suggests that there may be a correlation between the coital attitude and structure, as suggested by Abbott (2).

In this regard, it is interesting to note that in *Xiphidria* (Chalastogastra Orthandria) the copulation is dorsal (100), there being no transverse rotation of the appendages in this group (17), although in at least some of the Strophandria, the males and females mate while facing in opposite directions (17, 55, 100).

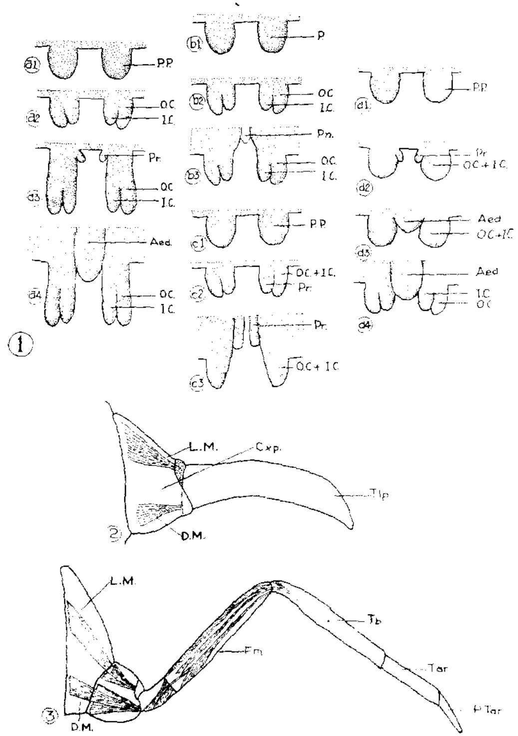
Specimens of the ichneumonid, *Angitia fenestralis*, taken *in copula*, were examined. In this species, the distal parts of the outer claspers grip the lateral portions of the subgenital plate of the female (Fig. 12), who no doubt extrudes her gonopore at the same time. The volsellae are rotated into the horizontal plane, owing to the muscular, ventro-posterior exsertion of the aedeagus and the increase in the blood pressure. The distivolsellae are bent upright through the contraction of muscle Q and the volsellar pincers are directed dorsally in respect to the male (but ventrally and slightly anteriorly with reference to the female). In this position, the pincers of the volsella are closed upon the intersegmental membrane of the female, owing to either the gonolacinial muscles or to the exserted aedeagus pulling the base of the gonolacinia posteriorly. The function of the volsellae is apparently to seize the conjunctival membrane, keeping it taut so that the aedeagus can be inserted into the female gonopore and be retained there. The aedeagus is exserted with its apex directed ventrally and, as it is strongly curved ventrally in this species, both its shape and its position at right angles to the main axis of the genitalia assist to retain the aedeagus in position during copulation. It should be noted that the function of the volsella was not definitely shown by dissections, because of the relaxations of the muscles but these conclusions were strongly indicated.

(To be concluded in next issue.)

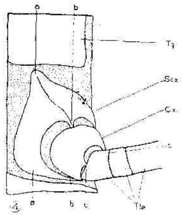
Abbreviations Used in the Illustrations

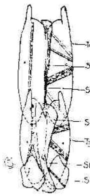
The abdominal segments are numbered with Roman numerals. The muscles of the male genitalia are lettered from A to V in accordance with the system of Boulangé (17) for the Chalastogastra.

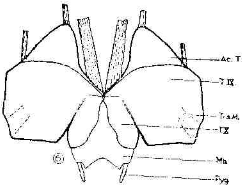
Ac.	antecosta	Ov.	ovipositor
Aed.	aedeagus	Ov. S.	ovipositor sheathe
Ast.	acrosternite	Pn.	penis
Bv.	basivolsella	P.P.	primary papilla
Bv. A.	basivolsellar apodeme	Pp.	parapenes
Cx.	coxa	Pr.	paramere
Cxp.	coxopodite	Ptar.	pretarsus
D.A.	dorsal area of the basivolsella	Pyg.	pygopod
D.M.	depressor muscle of telopodite	Scx.	subcoxa
Dv.	distivolsella	Snt.	syntergum
Ejac. D.	ejaculatory duct	Sntt.	syntergite
Erg.	ergot	Sp.	spiculum
Fm.	femur	S. Pl.	sensory plate
Gc.	gonocardo	Spt.	spatha
Gf.	gonoforceps	S-s.	intersternal
Gl.	gonolacinia	St.	sternum
Gsq.	gonosquama	Tar.	tarsus
Gst.	gonostipes	Tb.	tibia
Gst. A.	gonostipital arm	Tg.	tergum
Gtt.	gonotergite	Tlp.	telopodite
I.C.	inner clasper	T-s.	tergo-sternal
L.M.	levator muscle of the telopodite	T-t.	inter-tergal
M.	muscle	V.A.	ventral area of the basivolsella
Mb.	membrane	V.R.	ventral ridge of the aedeagus
O.C.	outer clasper	V. Str.	basivolsellar strut

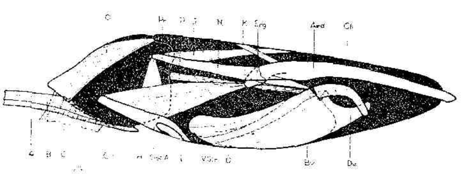


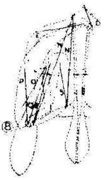
FIGS. 1–3. *Diagrams showing the developmental stages of the male genitalia in: (a) Sirex (according to Boulangé (17) and Vespa (Boulangé, after Zander (138). (b) Apis mellifica (according to Snodgrass (120), after Zander (138). (c) Apis mellifica (according to Michaelis (63). (d) Bombus (according to Boulangé, after Michaelis (63). 2. Diagram of the coxopodite and telopodite of a primitive limb, showing the levator and depressor muscles of the telopodite (after Snodgrass (124). 3. Diagram of a secondarily divided limb, showing the development of the secondary muscles (after Snodgrass (121, 124).*

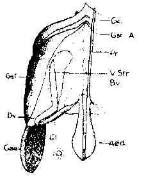


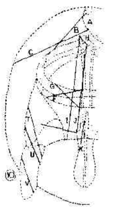


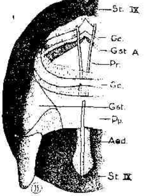




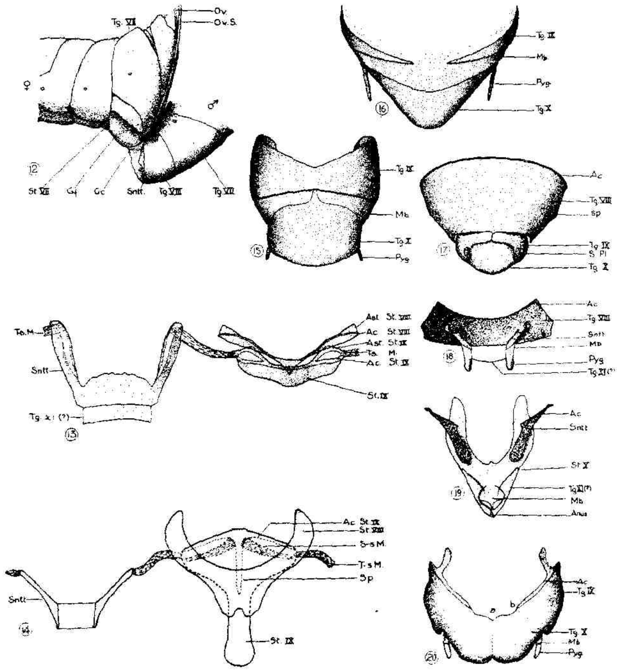




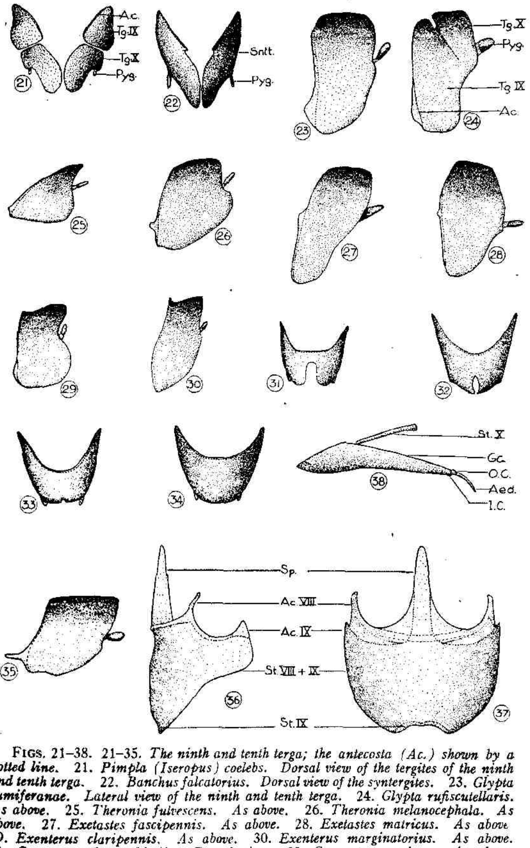




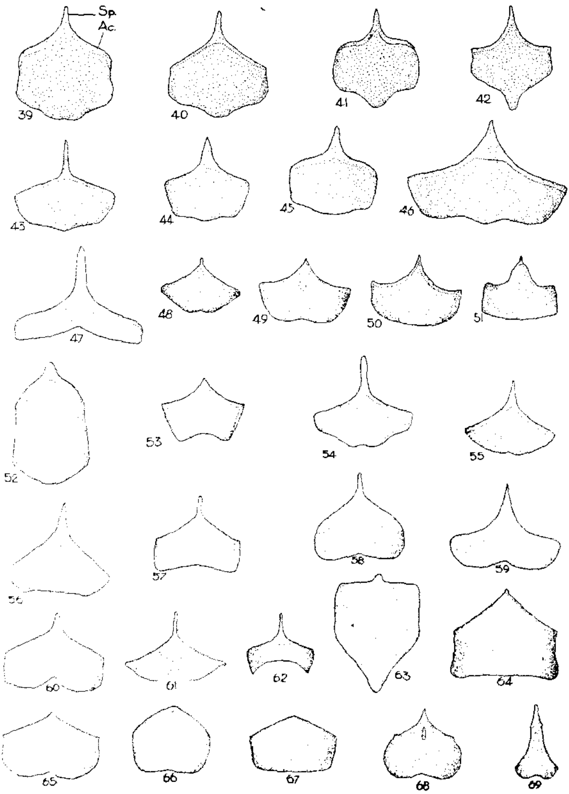
FIGS. 4–11. 4. *Diagram of a thoracic limb, illustrating the subcoxa, coxa and telopodite, together with their basal axes (a-a, b-b and c-c respectively) about which they move (modified after Snodgrass (122).* 5. *Megarhyssa lunator. Ventral view of abdominal segments VII-X, the terga spread horizontally and the left side of each sternum removed; tergal muscles placed to the left of the stippled sterna, the sternal and tergo-sternal muscles to the right.* 6. *Megarhyssa lunator. Dorsal view of the syntergites and their muscles, the syntergites placed in the horizonal plane.* 7. *Megarhyssa lunator. Inner aspect of the right half of the genital appendages, showing the muscles (exclusive of muscles D, E, F and L).* 8–11. *Diagrams of the genitalia and genital muscles in the Chalastogastra.* 8. *Ventral view of the genital muscles, the volsella placed in the horizontal plane; sclerites shown by broken lines and muscles by solid lines (redrawn after Boulangé, 1924).* 9. *Sclerites in Fig. 8 shown in relief; membranous areas shown by broken lines.* 10. *As in Fig. 8, but dorsal.* 11. *As in Fig. 9, but dorsal.*



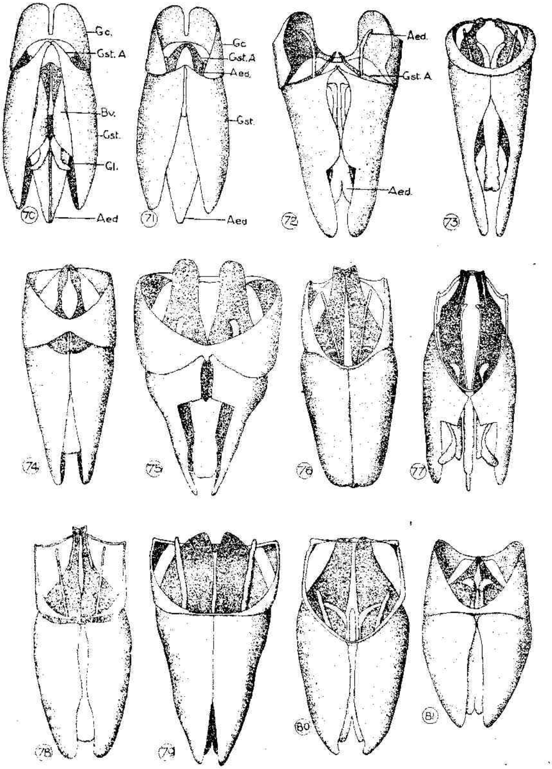
FIGS. 12–20. 12. *Angitia fenestralis. Lateral view of the terminal abdominal segments during copulation.* 13, 14. *External view of the syntergites, the eighth and ninth sterna, and the connecting muscles; stippled to show the degree of sclerotization.* 13. *Halictus lerouxii (Apoidea).* 14. *Andrena wilkella (Apoidea).* 15. *Cephus cinctus (Cephidae). Dorsal view of the terminal abdominal segments.* 16. *Pteronidea ribesii (Tenthredinidae). As above.* 17. *Brachymeria intermedia (Chalcididae). As above.* 18. *Lasius niger (Formicidae). As above.* 19. *Vespa maculata (Vespidae). As above.* 20. *Exeristes roborator (Ichneumonidae). Dorsal view of the syntergum, showing the line of median fission in the tenth tergum (a), and the line of inter-tergal fusion (b).*



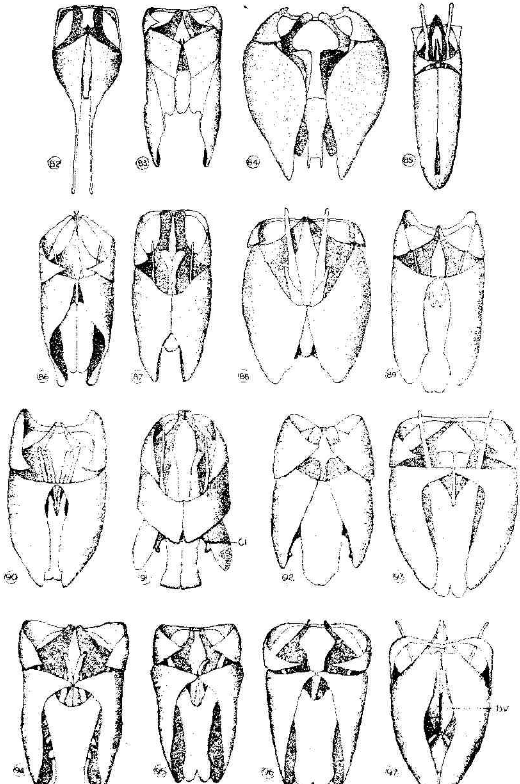
FIGS. 21–38. 21–35. *The ninth and tenth terga; the antecosta (Ac.) shown by a dotted line.* 21. ***Pimpla*** *(Iseropus) coelebs. Dorsal view of the tergites of the ninth and tenth terga.* 22. *Banchus falcatorius. Dorsal view of the syntergites.* 23. *Glypta* ***fumiferanae.*** *Lateral view of the ninth and tenth terga.* 24. *Glypta rufiscutellaris. As above.* 25. *Theronia fulvescens. As above.* 26. *Theronia melanocephala. As above.* 27. *Exetastes fascipennis. As above.* 28. *Exetastes matricus. As above.* 29. ***Exenterus*** *claripennis. As above.* 30. *Exenterus marginatorius. As above.* 31. ***Cremastus*** *flavo-orbitalis. Dorsal view.* 32. *Cremastus geminus. As above.* 33. ***Cremastus minor.*** *As above.* 34. *Cremastus incompletus. As above.* 35. *Ito-****plectis conquisitor.*** *Lateral view.* 36. *Vespa maculata (Vespidae). Lateral view of* ***hypandrium.*** 37. ***Vespa*** *maculata (Vespidae). Ventral view of* ***hypandrium.*** 38. ***Brachymeria intermedia*** *(Chalcididae). Lateral view of the male genitalia and* ***the tenth sternum.***



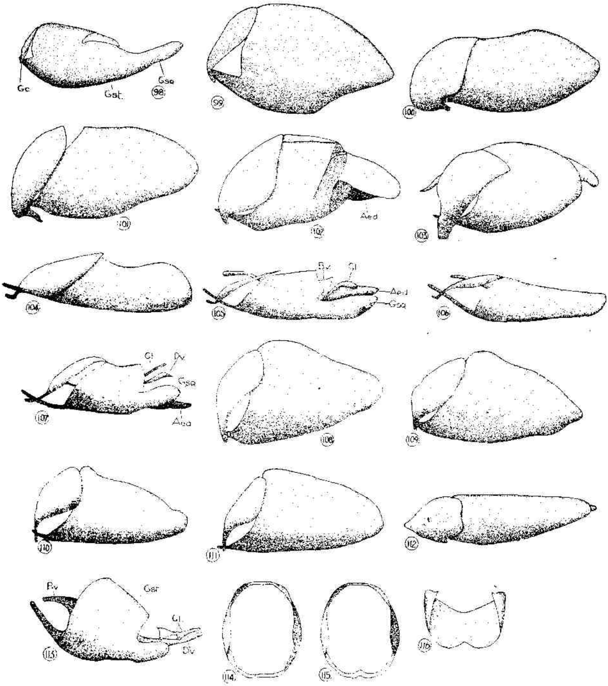
FIGS. 39–69. *Ventral view of the ninth sternum; membranous areas shown by broken lines.* 39. *Ichneumon variegatus.* 40. *Ichneumon animosus.* 41. *Ichneumon perscrutator.* 42. *Ichneumon grotei.* 43. *Phaeogenes gaspesensis.* 44. *Phaeogenes hariolus.* 45. *Hemiteles hemipterus.* 46. *Hemiteles subzonatus.* 47. *Hemiteles fulvipes.* 48. *Exetastes fascipennis.* 49. *Exetastes matricus.* 50. *Glypta rufiscutellaris.* 51. *Glypta fumiferanae.* 52. *Theronia fulvescens.* 53. *Theronia melanocephala.* 54. *Omorgus borealis.* 55. *Omorgus ensator.* 56. *Omorgus mutabilis.* 57. *Cremastus incompletus.* 58. *Cremastus flavo-orbitalis.* 59. *Cremastus minor.* 60. *Cremastus geminus.* 61. *Cremastus interruptor.* 62. *Pimpla coelebs.* 63. *Pimpla instigator.* 64. *Exenterus claripennis.* 65. *Exenterus canadensis.* 66. *Exenterus marginatorius.* 67. *Exenterus lepidus.* 68. *Tryphon incestus.* 69. *Megarhyssa lunator.*



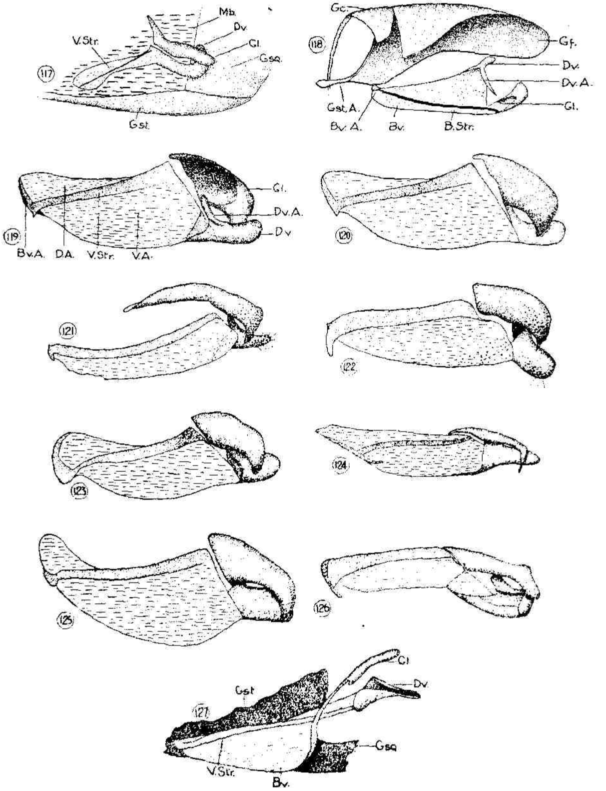
FIGS. 70–81. *The genitalia.* 70. *Megarhyssa lunator. Ventral view.* 71. ***Megarhyssa lunator.*** *Dorsal view.* 72. *Banchus falcatorius. As above.* 73. *Ophion obscurus. As above.* 74. *Agrypon flaveolatum. As above.* 75. *Exochilum circumflexum. As above.* 76. ***Cremastus minor.*** *As above.* 77. *Cremastus geminus. As above.* 78. *Cremastus flavo-orbitalis. As above.* 79. *Pristomerus vulnerator. As above.* 80. *Demophorus* ***robustus.*** *As above.* 81. *Orthopelma luteator. As above.*



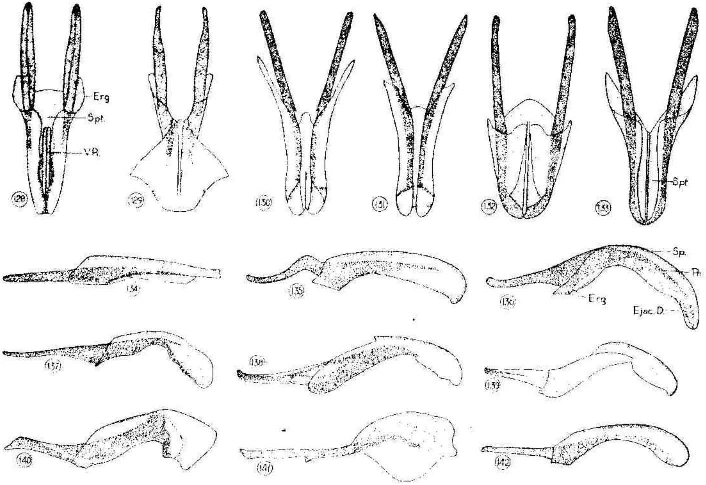
FIGS. 82–97. *The genitalia.* 82. *Mesochorus pectoralis. Dorsal view.* 83. *Hemiteles fulvipes. As above.* 84. *Hemiteles submarginatus. As above.* 85. *Enicospilus ramidulus. As above.* 86. *Apechthis ontario. As above.* 87. *Theronia fulvescens. As above.* 88. *Theronia melanocephala. As above.* 89. *Glypta rufiscutellaris. As above.* 90. *Glypta fumiferanae. As above.* 91. *Ephialtes tuberculatus. As above.* 92. *Ephialtes grapholithae. As above.* 93. *Exenterus canadensis. As above.* 94. *Exenterus claripennis. As above.* 95. *Exenterus lepidus. As above.* 96. *Exenterus marginatorius. As above.* 97. *Ichneumon longulus. Ventral view, showing the basal fusion of the volsella to the gonostipes.*



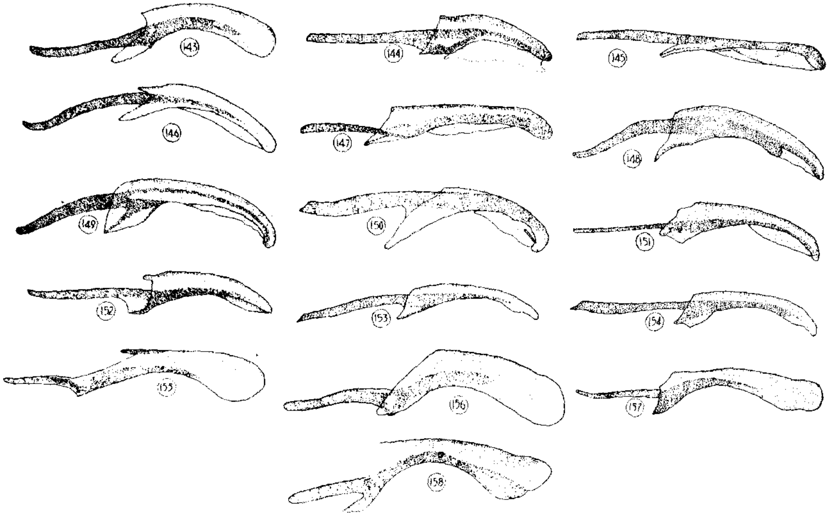
FIGS. 98–116. 98. *Hemiteles fulvipes*. *Lateral view of the genitalia*. 99. *Hemiteles submarginatus*. *As above*. 100. *Glypta rufiscutellaris*. *As above*. 101. *Glypta fumiferanae*. *As above*. 102. *Ephialtes tuberculatus*. *As above*. 103. *Ephialtes grapholithae*. *As above*. 104. *Cremastus interruptor*. *As above*. 105. *Cremastus geminus*. *As above*. 106. *Cremastus minor*. *As above*. 107. *Cremastus incompletus*. *As above*. 108. *Exenterus canadensis*. *As above*. 109. *Exenterus claripennis*. *As above*. 110. *Exenterus lepidus*. *As above*. 111. *Exenterus marginatorius*. *As above*. 112. *Megarhyssa lunator*. *As above*. 113. *Oryssus sayi* (*Oryssidae*). *As above, but the gonocardo absent*. 114. *Ichneumon longulus*. *Posterior view of gonocardo*. 115. *Ichneumon variegatus*. *As above*. 116. *Megarhyssa citraria*. *Dorsal view of gonocardo*.



FIGS. 117–127. *The volsella.* 117. *Tenthredella mesomelas (Tenthredinidae). Ventral view of the volsella in the horizontal plane and the adjacent sclerites (redrawn and shaded after Boulangé, (17).* 118. *Dolerus similis. Internal view of the widely separated volsella and gonoforceps.* 119. *Neotypus americanus. Lateral or internal view.* 120. *Neotypus americanus. Mesal or external view.* 121. *Ichneumon grotei. As above but showing portion of the basivolsella, torn from the gonostipes.* 122. *Ichneumon animosus. External view.* 123. *Hemiteles hemipterus. As above.* 124. *Enicospilus ramidulus. As above.* 125. *Cryptus annulatus. As above.* 126. *Cremastus minor. As above.* 127. *Cremastus incompletus. As above but showing the adjacent parts of the gonoforceps.*



FIGS. 128–142. *The aedeagus.* 128. *Megarhyssa lunator.* *Ventral view.* 129. *Agrypon flaveolatum. As above.* 130. *Glypta fumiferanae. As above.* 131. *Glypta rufiscutellaris. As above.* 132. *Theronia fulvescens. As above.* 133. *Theronia melanocephala. As above.* 134. *Megarhyssa lunator. Lateral view.* 135. *Ichneumon perscrutator. As above.* 136. *Ichneumon grotei. As above.* 137. *Ichneumon variegatus. As above.* 138. *Phaeogenes gaspesensis. As above.* 139. *Phaeogenes hariolus. As above.* 140. *Exochilum circumflexum. As above.* 141. *Agrypon flaveolatum. As above.* 142. *Glypta fumiferanae. As above.*



FIGS. 143–158. *Lateral views of the aedeagus.* 143. *Glypta rufiscutellaris.* 144. *Theronia fulvescens.* 145. *Theronia melanocephala.* 146. *Ephialtes grapholithae.* 147. *Ephialtes tuberculatus.* 148. *Pimpla brevicornis.* 149. *Pimpla coelebs.* 150. *Pimpla detrita.* 151. *Pimpla instigator.* 152. *Itoplectis conquisitor.* 153. *Itoplectis obesus.* 154. *Itoplectis pedalis.* 155. *Exenterus canadensis.* 156. *Exenterus claripennis.* 157. *Exenterus lepidus.* 158. *Exenterus marginatorius.*

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 15, SEC. D. DECEMBER, 1937 NUMBER 12

THE MALE GENITALIA IN THE HYMENOPTERA (INSECTA), ESPECIALLY THE FAMILY ICHNEUMONIDAE[1]

II. TAXONOMY OF THE ICHNEUMONID GENITALIA

BY OSWALD PECK

Introduction

During the last twenty-five years, there has been considerable disagreement upon the identity of the type species of some of the early ichneumonid genera and, therefore, upon their correct generic names (28, 31, 73, 103, 104, 134, 136). The merits of some of these proposed names are at present under the consideration of the International Commission upon Zoological Nomenclature (125); the names used in this study are therefore those consecrated by time and employed by Schmiedeknecht (114–117). This is of advantage also in that the majority of the species studied in this paper are European.

The classification of the Ichneumonidae is also still unsettled. The most recent complete classification is that of Schmiedeknecht (114). This has been brought further into line with recent thought by his unfinished series of fascicles, supplementing his Opuscula Ichneumonologica; in these he has adopted some of the concepts of Cushman and Rohwer (32), dividing the tribe Pimplini (*s.l.*) (116).

The generic limits of many ichneumonid genera are very indefinite at the present time and this condition is clearly shown in this study, where both holarctic and nearctic species are used. As an example, the nearctic species of the genus *Ichneumon* L. (*i.e.*, *Amblyteles* Cush. *et al.*) frequently cannot be placed with certainty within any of the genera or subgenera recognized by European workers (30, 81), although Cresson (25) attempted to do this. Cushman's concept of the genus *Ichneumon* has been retained. Too, the genus *Hemiteles* of Schmiedeknecht (114, 115) was considered by him as a single unit, for the sake of convenience. It is a very large group, susceptible to subdivision, as shown by Roman (102) and Cushman (30). Similarly the genus *Pimpla*, as recognized by Schmiedeknecht (114), is now divided into a number of subgenera or genera (30, 116).

When possible, the type genus of each tribe was selected for study. While, from the systematic viewpoint, the type genus should be studied whenever possible, yet, when material is scarce, the substitution of another genus may not be always detrimental and, in some cases, even advantageous. The type genus was arbitrarily created the nomenclatorial representative of a higher

[1] *Concluded from November issue.*

group, although possibly atypical. The type species is the systematic representative of a group but that is no criterion of its possession of the morphological attributes primitively common to the majority of that group. While in theory the characteristics of a group should be shown by a graduated series, in practice such thoroughness is often limited by time, material, and expense in publication.

These limitations have prevented any close study of specific differences, so that variations termed specific may prove to be only intra-specific. The practical restraints of material also preclude the thorough testing of any taxonomic inferences, therefore suggestions of this nature must be regarded as *purely tentative*, requiring the evaluation of the intensive systematist. This is needed also because the author claims no intimate knowledge of the various groups within the ichneumonids.

In order to aid the systematist to evaluate the data presented in this paper, the names of the ichneumonid species studied are listed below, the genera and species being grouped alphabetically in their respective tribes and subfamilies as recognized by Schmiedeknecht (114), subject to his revision of the Pimplinae (115). Cushman's division of the Pimplini (29) has also been indicated. Since the name *Ephialtes* L. is here used in the traditional sense for certain members of the Pimplini, all species of *Ephialtes* Cush. are referred to by his subgeneric name *Itoplectis*, here used with generic status merely to avoid a confusion in names.

Ichneumonidae

1. *ICHNEUMONINAE*
   - A. Joppini
     - *Trogus lutorius Fabr.*
   - B. Ichneumonini
     - *Ichneumon animosus* Cress.; *Ichneumon grotei* Cress.; *Ichneumon longulus* Cress.; *Ichneumon perscrutator* Wsm.; *Ichneumon variegatus* Cress.; *Neotypus americanus* Cush.
   - C. Phaeogenini
     - *Phaeogenes gaspesensis* Prov.; *Phaeogenes hariolus* Cress.
   - D. Alomyini
     - *Alomyia debellator* Fabr.
2. *CRYPTINAE*
   - A. Stilpnini
     - *Atractodes* sp.
   - B. Phygadeuonini
     - *Glyphicnemis crassipes* Prov.; *Microcryptus basizonius* Grav.; *Neostricklandia sericata* Cush.; *Stylocryptus subclavatus* Say.
   - C. Hemitelini
     - *Cecidonomus inimicus* Grav.; *Hemiteles (Astomaspis) fulvipes* Grav.; *Hemiteles (Astomaspis) submarginatus* Bridgm.; *Hemiteles (Hemiteles) hemipterus* Fabr.; *Hemiteles (Hemiteles) subzonatus* Grav.

D. Pezomachini

No representative.

E. Cryptini (including Mesostenini)

Acrornicus junceus Cress.; *Cryptus sexannulatus* Gr.

3. *PIMPLINAE*

A. Lissonotini

Cylloceria sexlineata Say; *Lissonota varia* Cress.

B. Glyptini

Glypta fumiferanae Vier.; *Glypta rufiscutellaris* Cress.

C. Lycorini

No representative.

D. Phytodietini

Phytodietus annulatus Prov.

E. Theroniini

Theronia fulvescens Cress.; *Theronia melanocephala* Cress.

F. Polysphinctini

Polysphincta sp. (*P. venator* De Gant?); *Zatypota percontatoria* Grav.

G. Labenini

No representative.

H. Rhyssini

Megarhyssa citraria Ol.; *Megarhyssa greenei* Vier.; *Megarhyssa lunator* Fabr.; *Rhyssa persuasoria* L.

I. Acoenitini

Phaenolobus arator Rossi

J. Xoridini

Deuteroxoides vittifrons Cress.

K. Odontomerini

Odontomerus canadensis Prov.; *Odontomerus pinetorum* Thoms.

L. Pimplini

(a. Pimplini Cush.)

Ephialtes (Calliephialtes) grapholithae Cress.; *Ephialtes tuberculatus* auctt. Angl. nec Fourc.; *Exeristes roborator* Fabr.; *Pimpla brevicornis* Grav.; *Pimpla (Iseropus) coelebs* Walsh; *Pimpla detrita* Holmgr.; *Pimpla examinator* Fabr.; *Pimpla instigator* Fabr.

(b. Ephialtini Cush. and Roh., nec auctt.)

Apechthis ontario Say; *Itoplectis* (*Ephialtes* Cush.) *pedalis* Cress.; *Itoplectis (Itoplectis) conquisitor* Say; *Itoplectis (Itoplectis) obesus* Cush.

4. *TRYPHONINAE*

A. Mesoleptini

Hadrodactylus typhae Geoffr.; *Lagarotus insolens* Grav.; *Lophyroplectus luteator* Thunb.; *Mesoleius multicolor* Grav.; *Mesoleius tenthredinidis* Morl.; *Perilissus (Spanotecnus) filicornis* Grav.

B. Tryphonini

Erromenus crassus Cress.; *Tryphon incestus* Holmgr.

C. Ctenisclni

Exenterus canadensis Prov.; *Exenterus claripennis* Thoms.; *Exenterus lepidus* Holmgr.; *Exenterus marginatorius* Fabr.

D. Paniscini

No representative.

E. Bassini

Bassus tricinctus Grav.; *Homotropus pectoratorius* Grav.

F. Orthocentrini

Orthocentrus sp.

G. Exochini

Exochus sp.; *Triclistus curvator* Fabr.

H. Metopiini

Metopius sp.

5. *OPHIONINAE*

A. Ophionini

Enicospilus ramidulus Grav.; *Ophion obscurus* Fabr.

B. Nototrachini

Nototrachys foliator Fabr.

C. Ophionellini

Hymenopharsalia foutsi Cush.

D. Anomalonini

Agrypon flaveolatum Grav.; *Anomalon flavifrons* Grav.; *Exochilum circumflexum* L.

E. Campoplegini

Diocles obliteratus Cress.; *Inareolata punctoria* Rom.; *Omorgus borealis* Zett.; *Omorgus ensator* Grav.; *Omorgus mutabilis* Holmgr.

F. Cremastini

Cremastus (Cremastus) flavo-orbitalis Cam.; *Cremastus (Cremastus) geminus* Grav.; *Cremastus (Cremastus) interruptor* Grav.; *Cremastus (Cremastus) minor* Cush.; *Cremastus (Zaleptopygus) incompletus* Prov.; *Demophorus robustus* Brischke; *Pristomerus vulnerator* Panz.; *Pristomerus appalachianus* Vier.

G. Porizonini

Orthopelma luteator Grav.

H. Plectiscini

No representative.

I. Banchini

Banchus falcatorius Fabr.; *Exetastes fascipennis* Cress.; *Exetastes fornicator* Fabr.; *Exetastes matricus* Prov.

J. Mesochorini

Mesochorus pectoralis Ratz.; *Cidaphus occidentalis* Cush.

The Value of the Genitalia in the Order Hymenoptera

The male genitalia have been widely used for taxonomic purposes in the principal pterygote orders, including the Hymenoptera. Among the latter group these appendages have been invaluable in some aculeate families, although they have not been used extensively in the Chalastogastra and Ichneumonoidea.

The ignoring of the male genitalia in these lower groups does not seem to be due to a lack of suitable characters, for the diversity of form has been shown by both Crampton (22) and Boulangé (17). Crampton, however, drew no systematic conclusions and did not include any specific studies but, when his work is compared with that of Boulangé, it is evident that good specific characters occur in *Cimbex*, *Xiphidria*, *Tenthredo*, *Dolerus* and *Cephus*, a wide representation of the Chalastogastra. Boulangé (17) considered the aedeagus to be the best source of specific characteristics, and Benson (9) found the shape of the ninth sternum in the tenthredinid genus *Athalia* to be specific in value, while the gonosquamae and parapenes were used by Rohwer (99) to separate the tenthredinid genera, *Lagium* and *Zalagium*. Among the nearctic sawflies, the genitalia are characteristic in some genera of the Allantinae (107), in species groups of *Dolerus* (105) and in individual species in *Empria* (106).

The separation of the Oryssidae from the Chalastogastra is supported by the radical difference in the resting position of the genitalia, those of the former always being concealed (38, 98), while those of the latter are infrequently so.

Differences have been observed among the ichneumonids, more especially the elongate gonosquama of *Banchus*, *Hemiteles nanus* Grav., *Lathrolestes* Foerst. and *Parabates* Foerst. (82), as well as those of the Mesochorini (21, 82, 114). The genitalia of *Amblyteles* sp., *Cryptus* sp. *Ithneumon lineator* Fabr., *Pimpla roborator* Fabr., *Psilosage ephippium* Holmgr. (a tryphonine) and *Metopius dissectorius* Panz. are dissimilar (21), as are those of *Hemiteles hemipterus* Fabr., *Collyria calcitrator* Grav. and *Pimpla detrita* Holmgr. (109), while those of *Calliephialtes* sp. (26) and of *Mesostenus* sp. and *Tryphon* sp. (8) differ again from each other.

The workers upon the aculeate groups have used the male genitalia extensively, especially in the Apoidea (87), Colletidae (72, 91), Andrenidae (5, 48, 49, 92, 112); Megachilidae (67–69), Bombidae (59, 86, 94, 95), Pompilidae (89), Chrysididae (90), Vespidae (10, 58), Tiphiidae (3, 60), Mutillidae (64, 65, 88) and the Sphecoidea (77, 78, 83, 93). Most of these workers confined themselves to a study of the hypandrium and of the undissected genital parts and found that the hypandrium and the apices of the outer claspers, of the inner claspers and of the aedeagus were extremely valuable in the identification of species. The situation seems to be adequately summed up by Mickel (65), who states, in reference to the Mutillidae, that:—

"the genitalia of the male have proved of inestimable value in settling certain points, especially in groups of males that are superficially alike. It has been possible to determine that certain external characters were of specific value by using the genitalia as a criterion. On the other hand, certain groups

of males quite different in superficial appearance possess genitalia that are practically identical, so that these structures have been of assistance not only in the distinguishing of species, but also in determining the relationships of species, which is exceedingly important from the viewpoint of phylogeny."

As a consequence of the extensive specific differentiation, the occurrence of generic and supra-generic characters seems to be rare. Hagens (48) suggested such for groups among the Apoidea but they do not appear to be well substantiated by his data. Parker considered the posterior spines of the hypandrium in some Bemicini as generic, while the shape of the spatha served to distinguish all but two of the twelve American genera (77, 78). The presence of the pygopods has been used by both Saunders (112) and Radoszkowski (93) as the first step in separating the Sphecoids, although in the Lasiini (Formicidae) it is of generic interest (37). A marked similarity of the genitalia was noted among the Pseninae (Sphecidae) and among the Tiphiidae, although the apical portions are subject to specific variation (61). This similarity is true also of many other groups; for example, the gonoforcipes of *Andrena* and *Halictus* (Apoidea), as illustrated by Atwood (5), seem to form two very distinct groups, although they are closely related. There thus appear to be reasonable grounds for suspecting that generic and possibly super-generic characters are present in the ichneumonid genitalia. Such an occurrence may be true in the Sphecoidea, for a key to the group was made by Radoszkowski (93), using chiefly the form of the inner clasper, the parapenial area and the gonosquama. However, few species were used in this key.

The existence of these higher characters is suggested also by the work of Boulangé (17), who stated that the basal portions of the chalastogastrous genital sclerites were of greater value in determining the larger groups than were the apices, owing to the restraint placed upon differentiation by the muscular attachments. Should this be so, it is not surprising that few characters of the higher denominations have been found, since most of the workers cited appear to have studied the genitalia as an undissected unit.

In contrast to these views, Richards (94, 96) holds that (i) almost any sclerites are liable to differentiation in the genus *Bombus* and (ii) in each of the main divisions of the aculeate groups, specialization takes place through fusion and, as this may occur within a family or even within a genus, it is not possible at present to use the male genitalia to any great extent in classification. While these views are not diametrically opposed to those of Radoszkowski, yet they do suggest that Radoszkowski's may need considerable modification and possibly even rejection.

Considering the evaluation of the genitalia in other hymenopterous groups, it seems probable that among the Ichneumonidae (i) there are numerous specific differences, occurring chiefly among the apical portions of the sclerites, (ii) generic and suprageneric characters are rare, to be found probably in the basal parts of the sclerites and (iii) if the differences involve fusion, then they may be regarded as abnormally variable. The work of Ceballos (21) and Salt (109) shows that Berthoumieu (12) was incorrect in believing that there

are no significant differences in the ichneumonid genitalia, but the former workers make no suggestion as to the possible taxonomic value of these structures.

The purpose of the taxonomic portion of this paper is to estimate the amount of variation that occurs in the genitalia of the ichneumonid males. The differences may be of real practical value, the quoted findings of Mickel (65) upon these structures in the mutillids then being perhaps equally applicable to the ichneumonids. On the other hand, the ichneumonid genitalia may be merely concealed structures, their differences only separating groups or species that are already readily recognized. This practical evaluation of the genital structures, however, must be left to the intensive systematist, only the type of variation being shown in this paper.

The Genital and Postgenital Terga among the Ichneumonids

As shown above, the hymenopterous gonotergum slowly evolved from the form of the pregenital tergum, first as gonotergites and subsequently as either a syntergum or a pair of syntergites, the syntergites later degenerating. This series of modifications occurring gradually in the Hymenoptera suggests that these structures should possess characters of value in identifying large groups within the ichneumonids, although the conclusions of Richards (96) in regard to the fusion of sclerites in the aculeates suggest otherwise.

Within the Ichneumonidae, the gonotergum is apparently always divided and it may be fused to the tenth tergum; the latter may be split into a pair of lateral tergites, although there is no significant reduction in the size of the syntergites. Unfortunately, these changes are not linked to the larger groups, for the inherent tergal characters, if present, appear to be concealed or partially masked by other influences, these being probably either the depressed or compressed shape of the abdomen, or the variations in the proportions of the hypandrium and of the gonopods, or both.

When compared with the specific variation of the hypandrium and the genital appendages, the compression or depression of the abdomen may be considered as distinctly inherent and, if so, there should be a correlation between the gonotergal form and the extreme knife-edge type of compressed abdomen. Among the ten most suitable species of the latter type (taken without regard for taxonomic considerations), *Ophion obscurus*, *Enicospilus ramidulus* and *Anomalon flavifrons* (all closely related), as well as *Hymenopharsalia foutsi*, have synterga, while *Acrornicus junceus*, *Banchus falcatorius* and *Lophyroplectus luteator* have syntergites; *Cremastus* spp., *Agrypon flaveolatum* and *Exochilum circumflexum* have a syntergum that is partially separated medianly. Evidently, if abdominal shape is an inherent factor in the modification of the genital tergum, it is completely dominated by more superficial characters and can be ignored.

The specific characteristics of the ninth and tenth terga are found in (i) the general proportions, (ii) the degree of inter-tergal fusion and (iii) the degree of the division in the tenth tergum.

Proportional variations appear to be common, some being shown in the lateral views of species belonging to the genera *Glypta*, *Theronia*, *Exetastes* and *Exenterus* (Figs. 23–30).

The degree of fusion between the terga may vary specifically, as shown in the complete fusion in *Glypta fumiferanae*, although this is incomplete anteriorly in *G. rufiscutellaris* (Figs. 23, 24). The latter condition is found also in *Cecidonomus inimicus*, while the closely related *Hemiteles* species have the intertergal fusion completed.

The division of the tenth tergum is not always uniform within a genus. *Cremastus* has a series of species that includes synterga, almost completely separated syntergites and intermediate forms (Figs. 31–34). In *Itoplectis conquisitor* the syntergum is completely fused medianly but in *I. obesus* it is incompletely so, while the syntergites of *I. pedalis* have completely fused intersegmentally. Similarly a wide variation occurs in *Pimpla*, since *P. examinator* and *P. instigator* possess synterga, and *P. brevicornis* syntergites with incomplete intersegmental fusion, while *P. detrita* and *P. coelebs* have separate tergites in both terga (Fig. 21). *P. detrita*, however, has been placed in *Epiurus* by Roman, as cited by Salt (109).

Apart from the exceptions already noted, the terga in each genus are of one type only. It is possible to consider three of the exceptions as heterogeneous genera, for *Cremastus* and *Pristomerus* have been divided into subgenera (27) and Pimpla contains both internal and external parasitoids (129). *Glypta*, however, is now considered as a homogeneous group. While great tergal variation occurs within the Phygadeuonini, Hemitelini, Pimplini, Cremastini, Pristomerini and Mesoleptini, yet in other tribes the tergal structures appear to be fairly uniform. Among the 22 species of the Ichneumoninae and Cryptinae (including 14 genera), 19 possess complete syntergites; however, two others, *Cecidonomus inimicus* and *Glyphicnemis crassipes* have the intertergal fusion incomplete, while the ninth and tenth terga of *Stylocryptus subclavatus* are divided into separate tergites. The terga of the other three subfamilies are remarkably varied, although tribally they show a uniformity of structure, apart from the exceptions already mentioned. The data are insufficient to show distinctly that the terga may be inherently constant within a tribe but, if this is so, then such uniformity of structure is usually either partially or entirely masked by specific differences.

During the study of the gonotergum, variations in the antecosta were seen, the anterior process in some Pimplinae being especially conspicuous. Since the antecosta is an internal ridge, serving as a place of attachment for muscles, it is unlikely that this structure would be greatly modified by the minor changes in the genitalia. The antecosta may therefore be a guide to the higher relationships of the ichneumonids.

No deliberate comparisons were made between the pretergal and gonotergal antecostae, yet they appeared to be similar, as is to be expected.

The genital antecosta may be (i) *linear* in form, as in *Exenterus marginatorius* (Fig. 30) (ii) *ovate* near the ventral margin, as in *Glypta rufiscutellaris* (Fig. 24),

or (iii) *appendicular*, a somewhat quadrate process projecting anteriorly from near the ventral margin of the antecosta, as in *Exeristes roborator* (Fig. 20) and *Itoplectis conquisitor* (Fig. 35).

The tergal antecosta is linear throughout the Ichneumoninae, Cryptinae and Ophioninae, exclusive of a small process in *Hemiteles submarginatus* and an oval thickening in both *Mesochorus pectoratorius* and *Cidaphus occidentalis* (Mesochorini); the two latter exceptions stress the remoteness of the Mesochorini from the other tribes of the Ophioninae. *Exetastes*, too, is an exception, for in *E. matricus* and *E. fascipennis* the antecosta is lineo-ovate, although in *E. fornicator* it is linear; however, the genus is aberrant, being placed midway between the Ophioninae and the Pimplinae by Schmiedeknecht (117) and in the Lissonotini by Cushman (30).

The great uniformity of the antecosta among the Ichneumoninae, Cryptinae and Ophioninae is in strong contrast to the variation that occurs among the Pimplinae and Tryphoninae and emphasizes the heterogeneity occurring in the latter two.

Among the Pimplinae this diversity is indicated also by the primitively linear antecosta being characteristic of the Lissonotini, Odontomerini, Acoenitini and *Pimpla coelebs*, groups that are widely separated from each other. This suggests that they may be the primitive members of four different natural groups, more especially since these represent respectively the four original tribes of the Pimplinae, *i.e.*, the Lissonotini, Xoridini, Acoenitini and Pimplini as recognized by Gravenhorst (47), Holmgren (54), Ashmead (4) and Schmiedeknecht (114).

The ovate antecosta occurs in Glyptini and Phytodietini, while the pendant form is found in the Pimplini (*s.s.*), except *Pimpla coelebs*. The intermediate ovato-appendicular type occurs throughout the Theroniini, Polysphinctini, Rhyssini and Xoridini. The ovate forms suggest that the Glyptini and Phytodietini are moderately primitive, particularly since (i) strong lissonotine affinities have been noted in the Glyptini (Cushman and Rohwer (32)) and (ii) the Phytodietini have been grouped with the Lissonotini by Foerster(40), Ashmead (4) and Schmiedeknecht (114), although lately given tribal rank by Cushman and Rohwer (32) and Schmiedeknecht (116). Cushman and Rohwer (32), however, regard the Lissonotini and Glyptini as forming a group that is not at all closely related to the Phytodietini or to the rest of the subfamily, although akin to the Banchine *Exetastes*.

Cushman and Rohwer (32) also separate the Odontomerini from the Xoridini. *Odontomerus* spp. possess linear antecostae, while this structure is ovato-appendicular in *Deuteroxoides vittifrons*. Thus the tribe Odontomerini is more primitive than the Xoridini.

However, while the shape of the antecosta may assist in determining the tribal relationships within the Pimplinae, yet more data must be obtained before this structure is accurately evaluated in this group. The possibility of the antecosta being tribal in value is enhanced by its uniformity in the Ichneumoninae, Cryptinae and Ophioninae, the antecosta in the Tryphoninae also showing marked agreement with the taxonomic groupings.

Among the Tryphoninae the pendant antecosta occurs in the members of the Bassini, Metopiini and the Orthocentrini, although it was not seen in the Exochini. The first two tribes form the Tryphonides schizodonti and T. aspidopi respectively, while the latter two form the Tryphonides prosopi, these being supertribes recognized from early times by Holmgren (53), Morley (74) and Schmiedeknecht (114). The occurrence of a linear antecosta in *Exochus* sp. and an ovate one in *Triclistus curvator* (Exochini) perhaps indicates (i) that the Exochini are related to the Orthocentrini, being the more primitive portion of the supertribe, and (ii) that the antecosta is of tribal rather than supertribal value.

The majority of the Tryphoninae, however, occur in the Tryphonides homalopi, in which the antecosta varies from linear to lineo-ovate, the ovate form occurring rarely. Specific differences may occur, for in the four species of *Exenterus* studied, *E. canadensis* and *E. marginatorius* have an ovate antecosta, while *E. claripennis* and *E. lepidus* have lineo-ovate ones; it is difficult to know what weight to place upon these variations. However, it should be noted that the small number of Tryphoninae examined, together with the large number of subtribes recognized in this group does not justify any conclusions being drawn.

The same is perhaps true of all of the subfamilies, yet the shape of the antecosta in some groups is remarkably constant and does suggest that these basal structures may prove, upon closer study, to possess super-generic characteristics that are very seldom subject to masking by generic or specific variations. This possibility is augmented by the deplorable state of the taxonomy in this group, for, in view of this, it would indeed be surprising if the correlation between the antecostal form and the taxonomic opinions was higher, particularly since one cannot expect to be able to group the major divisions of any taxonomic group by employing merely one structure.

The Ichneumonid Hypandrium

As in most of the Hymenoptera, the posterior and lateral margins of the ichneumonid hypandrium is specific in value, this being shown in *Ichneumon*, *Phaeogenes*, *Hemiteles*, *Exetastes*, *Glypta*, *Theronia*, *Omorgus*, *Cremastus*, *Pimpla*, and *Exenterus* (Figs. 39–67). Desclerotization in the median line sometimes occurs, as in *Exetastes* spp., *Cremastus* spp. and in *Tryphon incestus* (Fig. 68). However, caution is advisable in the use of the shape of the hypandrium since intra-specific variations occur in *Exeristes roborator;* moreover, the curvature and outline of the ninth sternum can be altered by differences in the preparation of mounts. In *Megarhyssa*, there appeared to be no differences between the species, although the form in the genus is distinctive (Fig. 69).

While the greater portion of the hypandrium is specific in value, yet the antecosta with its median spiculum appears to be as significant as the tergal antecosta in revealing higher group characteristics, for there is a high negative correlation between the maximum width of the gonotergal antecosta and the

length of the spiculum—if the gonotergal antecosta is linear, the spiculum is long, and *vice versa*. This correlation is almost perfect in the Ichneumoninae, Cryptinae and Ophioninae, and is high in the other two subfamilies. Since the antecostae and spicula are only roughly classified, it is surprising that the correlation should be so high. In no case was a positive correlation found.

As a source of specific differences, the hypandrium appears to be the most promising of the sclerites in the genitalia and the genital segment, not only because it varies in form, but because many of its characteristics may be seen without dissection and because its large size usually permits a reasonably easy dissection to the novice.

The Ichneumonid Gonocardo

As noted above, the annular gonocardo is formed by the fusion of two lateral semicircular sclerites, each differentiated from the base of a gonopod. Among the ichneumonids these gonopodites are broadest ventro-laterally or laterally, their apices tapering towards the median line of the abdomen.

The ventral tips of these sclerites are almost invariably fused together as a narrow bridge. This area of juncture is always narrow, even when the gonocardo is unusually broad ventro-laterally, as in *Megarhyssa lunator* (Figs. 70, 71, 112) and *Banchus falcatorius* (Fig. 72). Medio-ventrally there is sometimes a small internal ridge or apophysis, presumably the remnant of a suture existing before the fusion was completed; these are noticeable in the more heavily sclerotized gonocardines.

The dorsal portion of these gonopodites varies greatly in shape. A complete annulus is typical of the Ichneumoninae and of the less specialized tribes among the Ophioninae, while in the other families the meso-dorsal apices are usually joined by membrane alone. Unfortunately these generalizations are not entirely true of all groups, for both *Neotypus americanus* (Ichneumoninae) and *Stylocryptus subclavatus* (Cryptinae) are exceptional. Furthermore, as these genera are typical of their respective subfamilies, it is likely that many exceptions will occur in groups intermediate to these larger groups.

An intergradation of these types also occurs in the Ophioninae. The gonocardo is completely annular in the less specialized tribes, including the Ophionini, Nototrachini, Ophionellini, Anomalonini, Campoplegini, Cremastini and Pristomerini. The species *Ophion obscurus*, *Agrypon flaveolatum* and *Exochilum circumflexum* (Figs. 73–75) may suggest a tendency for the primitive gonocardo to be medio-dorsally broad in the longitudinal direction, although this is not true of all, *Enicospilus ramidulus* (Fig. 85) having a fairly narrow dorsal bridge. The same region appears to be consistently very narrow in the intermediate tribes Cremastini and Pristomerini (Figs. 76–80) and to have degenerated into membrane among the highly specialized tribes, Banchini, Porizonini and Mesochorini (Figs. 72, 81, 82). It is of interest to note that the completely annulate gonocardo of *Hymenopharsalia* (Ophionellini) agrees with the position assigned to it by Cushman (29).

It should be noted that the Porizonini are represented only by *Orthopelma luteator*, the genus being aberrant and related to the Pimplinae (114), although placed in the Porizonini by both Schmiedeknecht (114) and Cushman (30). The remaining two specialized tribes, Mesochorini and Banchini, are also perhaps only placed provisionally in the Ophioninae. The Banchini may be related to the Lissonotini through the genus *Exetastes*, which is placed with the Banchini by Schmiedeknecht (114) and in the Lissonotini by Cushman (30). The remoteness of the genus has been emphasized by earlier taxonomists including Handlirsch (50), who created a subfamily for the genus; this attitude is supported by the occurrence of Braconid characteristics in the respiration of the banchine larvae (129). The Banchini have been regarded as remote, for Foerster (40) and Viereck (135) raised the group to family rank. The remaining group, the Mesochorini, is differentiated from almost all other Ichneumonidae by the extremely elongate outer claspers in the males (Fig. 82); the peculiarly flattened head and enlarged areolet are also highly characteristic. When the apparently consistent habit of hyperparasitism is also considered, it appears that the Mesochorini may merit elevation to the rank of supertribe.

Upon these grounds it is evident that the more specialized tribes of the Ophioninae possibly may not be true members of the subfamily and, if so, the Ophioninae are characterized by a completely annulated gonocardo. However there were not enough species available in these tribes for this to be determined.

While the gonocardo undoubtedly possesses characteristics common to large groups within the Ichneumonidae, yet this structure also shows definite specific differences. Viewed in cross-section, the ventral portion may be straight, as in *Ichneumon longulus* (Fig. 114) or incurved as in *I. variegatus* (Fig. 115). The gonocardo, when seen from below, frequently exhibits variation, the medio-ventral portion being either straight, curved anteriorly or curved posteriorly. The anterior curve is present in *Cremastus* spp., (Figs. 76–78) although the form of this portion of the gonocardo may vary specifically. Within *Ichneumon*, *Phaeogenes* and *Hemiteles (Astomaspis)* both the straight and the posteriorly curved forms occur, the former in *I. grotei* (barely curved), *P. hariolus* and *H. fulvipes* (Fig. 83) and the latter in the other *Ichneumon* spp. examined, in *P. gaspesensis* and in *H. submarginatus* (Fig. 84). In the examined species of *Omorgus* and *Exenterus* this area was invariably straight.

Among the Pimplinae also, the ventro-median portion of the gonocardo is of specific interest. *Megarhyssa citraria* is readily distinguished from the American species, *M. lunator* and *M. greenei*, by the antero-ventral notch in the former being but half the length and V-shaped without parallel sides (Figs. 70, 116). Within the genus *Pimpla*, too, this area possesses diagnostic characters. A prominent antero-median lobe distinguishes *P. brevicornis* from the other species that were examined. Some *Pimpla* species, including *P. examinator*, *P. instigator* and *P. detrita*, are exceptional among the ich-

neumonids, for the two lateral halves (*i.e.*, the basal gonopodites) have not fused ventrally; unfortunately, this has weakened the gonocardo, making it difficult for the unwarned to extract the genitalia without separating the two halves of the gonocardo. *Apechthis ontario* (Fig. 86) *Itoplectis conquisitor* and *I. obesus* are distinguished by the median portion being bent posteriorly as a broad "V" but this is not true of *I. pedalis.*

Yet, while the gonocardo may vary within a genus, the similarity of this structure in some genera, notably *Ichneumon*, *Megarhyssa*, *Glypta*, *Cremastus* and *Exenterus* suggest that these specific differences have not entirely concealed some characters of higher value.

The Ichneumonid Gonoforceps

The tribe Mesochorini is usually distinguished from other ichneumonids by its singular, extremely elongate gonosquamae, although this character is possessed also by *Ctenopelma sanguineum* Prov. (Tryphoninae) and by some or all of the Ophionellini. One might expect that the gonoforcipes could be of use in identifying some of the larger groups. This, however, seems highly improbable, for while individual groups sometimes possess a common facies, yet only rarely, as in *Megarhyssa* and the *Mesochorini*, does one find that such facies are peculiar to one group.

This infrequence is due, at least in part, to the fact that each part of the gonoforceps may be differentiated specifically. The form of the gonostipital arm may vary within a genus, as in *Cremastus*, *Hemiteles (Astomaspis)*, *Theronia*, *Glypta*, *Ephialtes* and *Exenterus* (Figs. 76–78, 83, 84, 87–96), as well as in *Ichneumon* and *Phaeogenes*. The main portion of the gonoforceps may vary in shape within a genus but seldom as radically as in *Ephialtes*, *Theronia* and *Exenterus*. The apex of the outer claspers often varies greatly within genera such as *Hemiteles*, *Glypta*, *Ephialtes*, *Cremastus* and *Exenterus* (Figs. 98–111).

The Ichneumonid Volsella

The ichneumonid volsella is a very flexible structure, this being necessitated by its function of finding, clasping and stretching the membrane lying adjacent to the female gonopore. Owing to this versatility of form in the individual, there seems to be no ideal method of mounting these structures without subjecting some part to possible distortion.

This part has been illustrated in several planes by Cushman (26), Becker (8), Ceballos (21) and Salt (109) and their works collectively show that it does vary considerably. However, as they dealt with thirteen species, representing eleven genera and all five of the traditional subfamilies, no taxonomic conclusions can be made from their work.

The typical ichneumonid volsella is similar to that of *Neotypus americanus* (Figs. 119, 120), the names of the parts being indicated in these figures. The area dorsal to the volsellar strut is termed, when present, the *dorsal area* of the volsella (D.A.), its margin almost invariably being supported anteriorly by the basivolsellar apodeme (Bv. A.).

While the volsella usually is completely separated by membrane from the gonoforceps, eleven exceptions were found, these representing four tribes in the Tryphoninae and one each in the Ichneumoninae, Cryptinae and Ophioninae. This character, therefore, appears to be widely spread, although intermittent. This peculiarity is evidently specific, at least in some groups, for a sclerotic tongue connects the antero-ventral part of the basivolsella to the gonostipes in *Ichneumon longulus* (Fig. 97), *I. grotei* and *I. variegatus*, although the volsella is free in *I. animosus* and in *I. perscrutator*. In the genus *Cremastus* also, *C. incompletus* and *C. geminus* have the volsella joined broadly to the gonostipes, although the other species examined in this genus have the volsella entirely separated. This latter condition is true also of *Neostricklandia sericata*, *Lagarotus insolens*, *Homotropus pectoratorius*, *Bassus tricinctus*, *Triclistus curvator* and *Metopius* sp. The fusion in the last species occurs for more than half of the total length of the volsella.

Among the Ichneumoninae, excepting *Neotypus americanus*, the basivolsellar apodeme is large, curving ventrally (Figs. 121, 122), and this is true also of the species of *Atractodes* (Stilpnini) examined. Among the Phygadeuonini the apodeme is small but among the more specialized Cryptinae, such as *Hemiteles hemipterus* (Fig. 123), the apodeme is large and extended dorsally. To this *Hemiteles subzonatus* is an exception; for the apodeme is small and appears to be ventro-lateral.

The size of the dorsal area is closely correlated with the dorsal growth of the basivolsellar apodeme, for it is absent in the five *Ichneumon* species, scarcely present in *Alomyia debellator* and narrow in the other Ichneumoninae, as well as in *Atractodes*. Among the Phygadeuonini, the dorsal area is moderate in size, while it is well developed in the more specialized Cryptinae, such as *Cryptus sexannulatus* (Fig. 125). In *Glyphicnemis crassipes* (Phygadeuonini), however, the apodeme is small and does not support the large dorsal area. While this correlation is true also of *Neotypus americanus* and *Hemiteles subzonatus*, the former has a large dorsal area and the latter none, thus disagreeing with their present taxonomic position. This suggests that there is a closer correlation between the form of the apodeme and that of the dorsal area than between the shape of these structures and the taxonomic grouping within the Ichneumoninae and Cryptinae.

This conclusion also appears to be applicable to the other subfamilies, although, again, each subfamily has a strong tendency towards a particular type of apodeme with its corresponding form of the dorsal area.

Among the Tryphoninae, the basivolsellar apodeme is usually weakly developed, although it is strongly curved laterally in *Triclistus curvator* and ventrally in *Erromenus crassus*.

In the Ophioninae the apodeme is invariably ventral. The dorsal area is usually absent, except in *Orthopelma luteator*, *Exetastes* spp. and *Enicospilus ramidulus*. The first species has the apodeme developed dorsally, while the dorsal area is large. However, as the genus *Orthopelma* is aberrant (114), this case may be disregarded. In *Exetastes* spp. the dorsal area is present,

although the apodeme is ventral and lateral; this genus is also aberrant (30, 114). In *Enicospilus ramidulus* (Fig. 124) the genitalia are unusually elongate so that this variation in volsellar form may be due to the need for reinforcement, for the apodeme is not of the normal dorsal type.

The form of the basivolsella divides the Pimplinae into two main groups, The Phytodietini being intermediate. The more specialized tribes are separated from the Lissonotini and Glyptini by the apodeme being well developed dorsally and by the great growth of the dorsal area. This agrees with the data upon the antecosta, which grouped together the Lissonotini and the Glyptini, as well as possibly the Phytodietini.

While the basivolsella is essentially a basal structure in which one may expect to find subfamily or tribal characters, yet it is also subject to generic or specific changes, not only in its fusion with the gonostipes, as already mentioned, but also in its proportions. The outline within some of the examined genera is similar, although specific differences may occur, as in *Ichneumon*. In this genus the narrowness of the basivolsella in *I. grotei* (Fig. 121) and *I. longulus* separates these species from *I. animosus* (Fig. 122), *I. variegatus* and *I. perscrutator*.

Among most of the Ichneumonidae, the apical portions of the volsella vary little from the type of *Neotypus americanus* (Figs. 119–120) although characters of specific value are to be found in the apex of the gonolacinia, for this may vary from being bluntly rounded, as in *Neotypus americanus* and *Glypta* spp. to being sharply pointed, as in *Ichneumon* spp. Within the genus *Ichneumon*, the length and shape of the gonolacinia varies, as shown in *I. grotei* (Fig. 121) and *I. animosus* (Fig. 122). A remarkable difference occurs in *Cremastus*, the gouolacinia being normal in both *C. minor* (Fig. 126) and *C. flavo-orbitalis*, although it is fused to the basivolsella in *C. incompletus* (Fig. 127) and *C. geminus*. This is of special interest as it emphasizes the fact that a character may appear to be extremely stable within the entire family although masked at times by a specific variation.

The Ichneumonid Aedeagus

The shape of the typical ichneumonid aedeagus is a weakly depressed cylinder, having the posterior apex curved somewhat ventrally and an elongate paramere extending anteriorly from each side. However, bizarre forms may occur within the family, as in *Agrypon flaveolatum* (Figs. 129, 141) and *Exochilum circumflexum* (Fig. 140).

The parameres are primitively joined to each other by membrane but the latter is often partially sclerotized, more particularly the dorsal or spathal area; this process is completed in some of the higher members of the Pimplinae, a medio-ventral fold (V.R.) being formed just in front of the apex (Figs. 70, 128, 134, 146–154). In most ichneumonids, however, this fold is membranous and allows the ventral portions of the valves to separate laterally. On this account the valves must be closed before comparisons are made in either the ventral or dorsal views.

The free anterior portion of the parameres is also subject to distortion, during treatment with potassium hydroxide, their shape therefore being of little taxonomic value. However, the anterior apex in *Hemiteles fulvipes* is dilated vertically, both upwards and downwards, in the form of a fishtail; in the other studied species of *Hemiteles* the paramere is rod-shaped, this being the almost invariable form in the Ichneumonidae. The length of the free portion may also vary specifically, as it is proportionately much less in *Ichneumon perscrutator* (Fig. 135) than in the other species studied in this genus (Figs. 136, 137).

These five species are readily divisible into three groups by their aedeagal shape. In both *I. grotei* (Fig. 137*) and *I. longulus* the aedeagus has small ergots and a dentate medio-ventral ridge, the ergots being long and the ridge without teeth in *I. variegatus* (Fig. 136), *I. animosus* and *I. perscrutator* (Fig. 135). The latter is readily distinguished from the others by the less falcate appearance and the shortness of the parameres.

All parts of the aedeagus seem to be differentiated specifically at times, as can be readily seen in *Hemiteles, Ichneumon, Phaeogenes, Glypta, Theronia, Ephialtes, Pimpla, Itoplectis* and *Exenterus* (Figs. 83–84, 130–133, 135–139, 142–158). The diverseness in form between species must conceal and offset to a very great extent any intrinsic character of higher value, although some of the species within a genus do appear to have a facies common to the aedeagus.

Summary

(1) The muscles of the male genitalia (and of the adjacent parts) in the ichneumonid, *Megarhyssa lunator* F., are shown to be homologous with those occurring in other hymenopterous groups, especially the Chalastogastra.

(2) The median genital organ is an aedeagus. The volsella and gonostipes are derived from the same primary part of the gonopod. The gonocardo may represent the fused coxopodites and the claspers the telopodite; the ontogenetic and myological data are not in agreement but the latter is probably much diminished in value by the genital muscles being aided by changes in blood pressure.

(3) The term *gonolacinia* should be used for the apical sclerite of the inner clasper, as a substitute for the word *sagitta*. The latter, used originally for structures now identified as parameres, is frequently misapplied.

(4) The ninth and tenth terga in the Chalastogastra are but little modified but those of the Aculeata are small because of their invagination. In the ichneumonids, the ninth is invariably divided into a pair of lateral tergites; these may be fused to the tenth tergum which may also be divided medianly.

(5) The ninth sternum in both the Chalastogastra and Ichneumonidae is large, possessing an antero-median appendage, the spiculum. In the higher Hymenoptera, the ninth sternum is small and sometimes fused to the eighth.

*The names *I. grotei* and *I. variegatus* are interchanged on the illustration on page 251.

(6) Among the Ichneumonidae, almost every part of the genitalia (and of the adjacent parts) may be differentiated specifically. Variations that appear of value in identifying groups almost invariably have exceptions. While some of the latter may be due to misplacements of groups or species, the number of exceptions shows that specific variations mask characters of higher value to such an extent that the latter are unreliable.

(7) The form of the antecosta in the ninth tergum and the ninth spiculum may prove to have some value in the grouping of tribes. Unfortunately, these parts are not available for observation without dissection and treatment.

Acknowledgments

The writer wishes to express his thanks to Dr. R. A. Cushman of the U.S. National Museum in Washington, D.C., who suggested that the ichneumonid genitalia in the male would prove to be a fertile field of investigation. For the granting of various facilities, the author is deeply indebted to Dr. W. H. Brittain of McGill University, Montreal, to Drs. A. Gibson and J. McDunnough of the Canadian Entomological Branch, Dominion Department of Agriculture, Ottawa, and to Dr. W. R. Thompson, superintendent of Farnham House Laboratory, Farnham Royal, Bucks., England.

The value of accurately determined material cannot be over-emphasized in a work of this kind, particularly since ichneumonids are frequently difficult to name. For the care and help in this difficult phase of the work special thanks are due to the personnel of the Farnham House Laboratory, to Mr. J. F. Perkins of the British Museum, to Messrs. G. S. Walley and L. F. Finlayson of the Canadian Entomological Service, to Dr. O. W. Richards of the Imperial College of Science, London, and to the Department of Entomology at Macdonald College, McGill University.

The author is deeply indebted to Dr. Thompson for his ready help. To him, as well as to Dr. W. H. Brittain and Dr. E. M. DuPorte of McGill University, the author wishes to acknowledge his gratitude, for their stimulating influence in problems of biological control and of morphology, for criticisms and for their kindly personal encouragement during the work.

Valuable advice in illustrating was given by Mr. R. J. Spittle of Farnham House. The lettering of the drawings was kindly done by Mr. F. C. Hennessey of the Entomological Branch, Ottawa. Thanks are also due to those workers who assisted either by criticizing the reasoning, by suggesting additional relevant facts, or by editing the text.

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STUDIES ON THE HETEROPHYID TREMATODE *APOPHALLUS VENUSTUS* (RANSOM, 1920) IN CANADA

PART III. FURTHER HOSTS[1]

BY THOMAS W. M. CAMERON[2]

Abstract

Amia calva and *Ictalurus punctatus* are added to the list of carriers of the cysts of *Apophallus venustus* in the lower Ottawa Valley, and a probable human infection with the adult trematode is recorded.

Fish Hosts

During the summer of 1937, work was continued on the life history of *Apophallus venustus* with the result that two new fish have to be added to the list of carriers of the metacercaria given in Part II. of this series (1). These fish are the "fresh-water dogfish", "bowfin" or "lush" *(Amia calva)* and the "channel-cat" or "spotted catfish" *(Ictalurus punctatus)*.

A number of specimens of both of those fish were caught by line in the Ottawa River, off Ste. Anne de Bellevue. As in the previous work, they were fed to cats which had been raised in the Institute and had not previously received any form of fish whatever.

The lush were not heavily infected but the catfish carried a considerable number of cysts. While it is not possible to give an exact comparison, they seem to be as suitable hosts as small-mouth black bass. This is of considerable interest as this species of catfish, like the bass, is an excellent food fish and a fair game fish. Various authorities rate it as being the most edible of fresh-water fish other than the Salmonidae.

Definitive Hosts

During the year, with the assistance of Dr. Angrove of the Ste. Anne de Bellevue Military Hospital, a considerable number of samples of human feces was submitted to the Institute for examination.

In one of these stools a number of eggs were found which agreed completely in shape and size with the eggs of *Apophallus venustus*. In spite of extensive search during the past three years no other trematodes of fish-eating mammals have yet been found in this neighborhood. Moreover, every species of this family of Trematodes, that has so far been tested in man, has found him a more or less suitable host.

While it is impossible to say conclusively that this patient was harboring some specimens of this parasite it is extremely probable that he was in fact doing so.

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[1] *Manuscript received October 29, 1937.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Quebec, with financial assistance from the National Research Council of Canada.

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